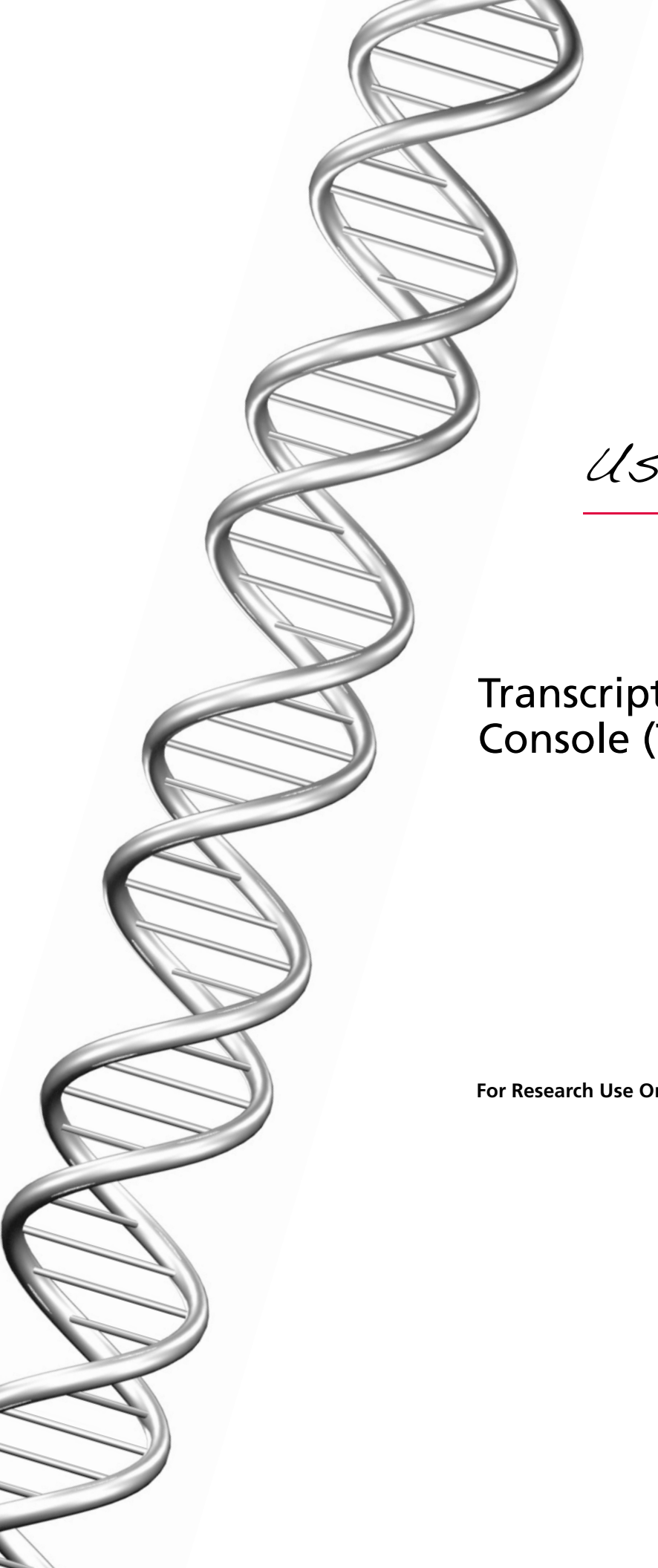

Affymetrix®

User Manual

Transcriptome Analysis Console (TAC) Software

For Research Use Only. Not for use in diagnostic procedures.

P/N 703150 Rev. 1

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Introduction

Transcriptome Analysis Console (TAC)

TAC performs statistical analysis to obtain a list of differentially expressed genes and alternative splicing events. IT also provides the visualization of genes, exons, junctions and transcript isoforms. TAC runs analysis based on Expression Console (EC) generated CHP files.

NOTE: Affymetrix recommends that if you are using GCOS CEL files, you should use the Data Transfer Tool (DTT) provided by Affymetrix to move the CEL files out of the GCOS directory.

Software and Hardware Requirements

The table below-shows the operating systems the recommended minimum requirements. (Table 1.1)

IMPORTANT: Larger data file sizes associated with Whole Transcriptome arrays should be taken into account when calculating the necessary available disk space requirement.

Larger data file sizes associated with Whole Transcriptome arrays should be taken into account when calculating the necessary available disk space requirement.

Table 1.1 System Requirements

64-bit Operating System	Speed	Memory (RAM)	Available Disk Space*	Web Browser
Microsoft Windows® 7 professional operating system with Service Pack 1	8 GHz Intel Pentium Quad Core Processor	16 GB RAM	150 GB HD + data storage	IE 7.0 and above
Microsoft Windows® XP operating system with Service Pack 2.0	4 GHz Intel Pentium Quad Core Processor	8 GB RAM	150 GB HD + data storage	IE 7.0 and above

Recommended: 16 GB of RAM.

Installation Instructions

To install the Transcriptome Analysis Console software:

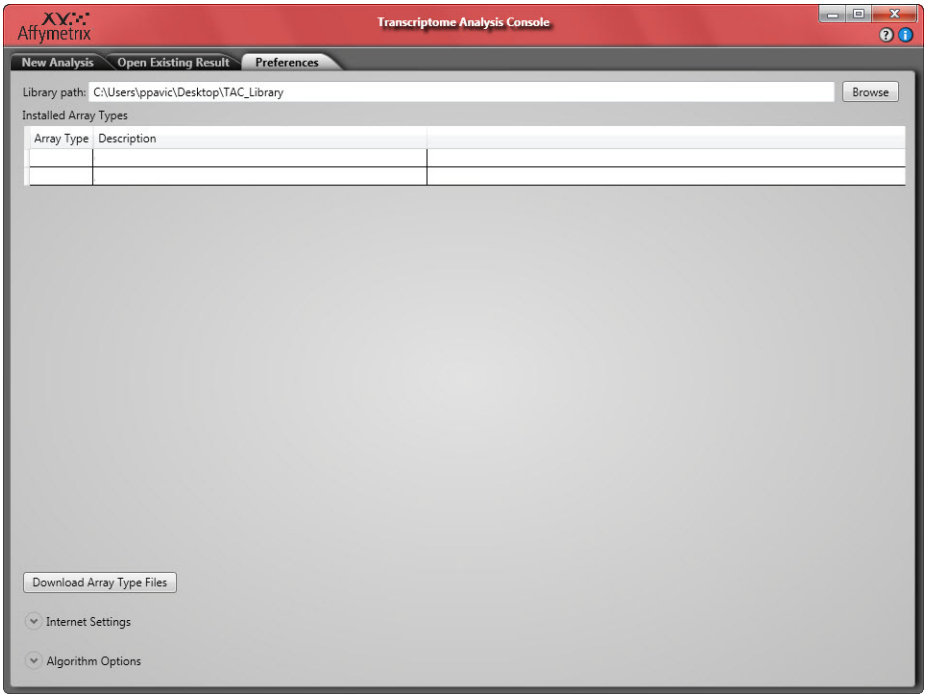
1. Go to **www.affymetrix.com** and navigate to the following location:
   Home > Products > Microarray Solutions > Instruments and Software > Software >
2. Locate and download the zipped TAC software package.
3. Unzip the file, then double-click **TAC64.exe** to install it.
4. Follow the directions provided by the installer.

The setup process includes and installs the required Microsoft component,.NET Framework 4 Client Profile.

Preferences Tab

Use the Preferences tab to setup or change a library path, download library and annotation files, and modify algorithm options. (Figure 1.1)

Figure 1.1 Main Preferences window

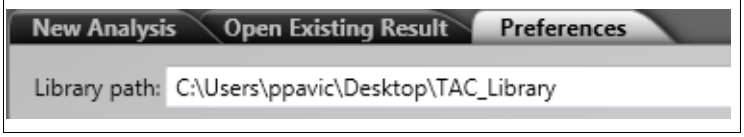


Parts of the Preferences Window

Setting Up a Library Path

IMPORTANT: The first time you install TAC, you must assign a path to your library folder. The library folder contains the library and annotation files required to run the TAC software.

Figure 1.2 Library Path

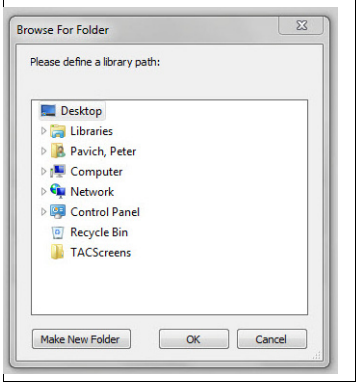


Do the following to create a new Library Path:(Figure 1.2)

1. Click Browse... (right side of library path) to create a new library folder path.

The following Browse For Folder window appears. (Figure 1.3)

Figure 1.3 Browse For Folder

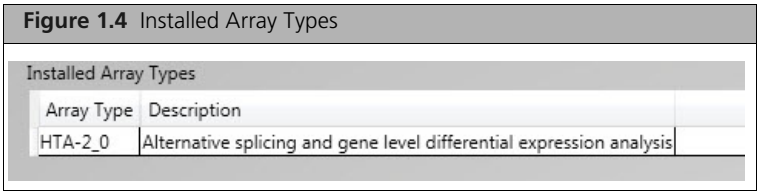


2. Click **New Folder** to choose a new Library folder. Name the new folder, then click **OK**.

Installed Array Types

Displays the currently installed array types inside your library folder. (Figure 1.4)

Figure 1.4 Installed Array Types



Download Array Type Files (Internet Connection Required)

1. Click **Download Array Type Files** to download library and annotation files from Affymetrix.com.

The **NetAffx Account Information** dialog window appears.

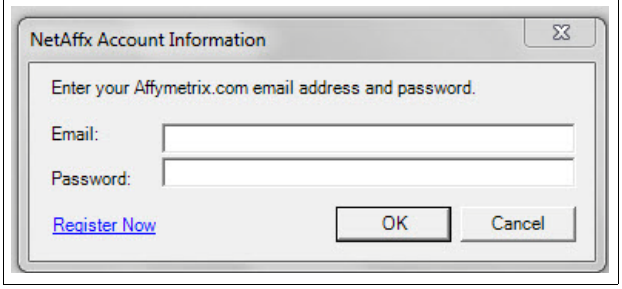
2. Enter your Netaffx account email and password, then click **OK** or click the **Register Now** link to obtain a new account.

Do the following to download library and/or annotation files:

1. Click Download Array Type Files.

The NetAffx Account Login window appears. (Figure 1.5)

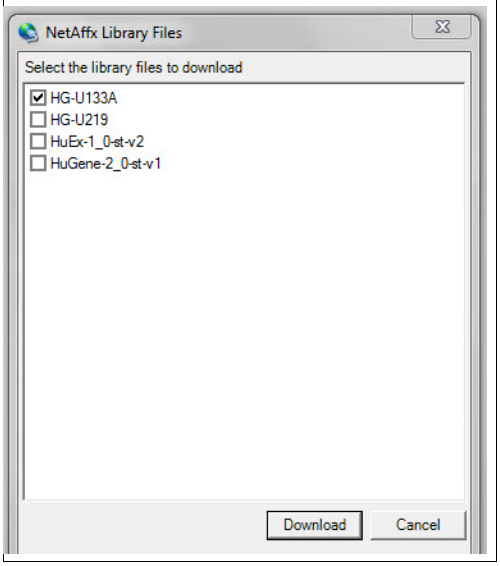
Figure 1.5 NetAffx Account Login



2. Enter your NetAffx email and password, then click **OK**.

The following window appears; (Figure 1.6)

Figure 1.6 NetAffx Library Files



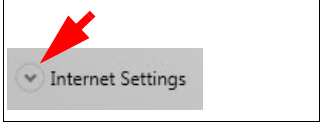
3. Click the checkbox next to the library file(s) you want to download, then click **Download**.

A progress bar appears, then the downloaded library and annotation file(s) appear in the **Installed Array Types** panel.

Internet Settings

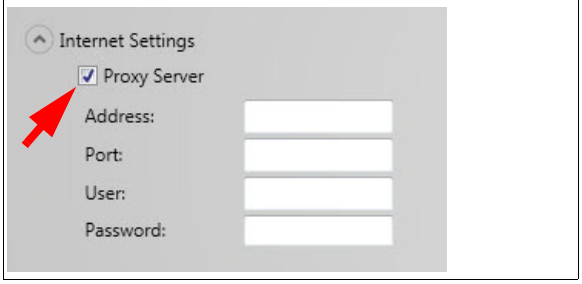
- To enable the Internet Settings option, click on the down-arrow button. (Figure 1.7)

Figure 1.7 Internet Settings



- If you are using a Proxy Server, click its checkbox, then complete the required fields. (Figure 1.8)

Figure 1.8 Internet Settings - Proxy Server Information



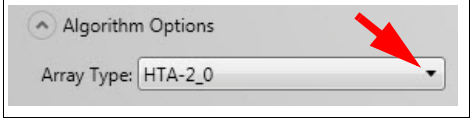
Algorithm Options

- To customize Algorithm Options, click on the down-arrow button. (Figure 1.9)

Figure 1.9 Algorithm Options Drop-down

If you have more than one array type installed and you want to change algorithm options for another array, use the drop-down menu to select a different array. (Figure 1.10)

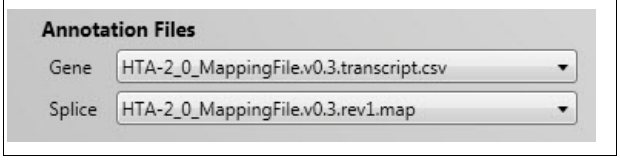
Figure 1.10 Algorithm Options



Annotation Files

Use the drop-down menus to select different versions of annotation files for various types of analysis. (Figure 1.11)

Figure 1.11 Annotation Files

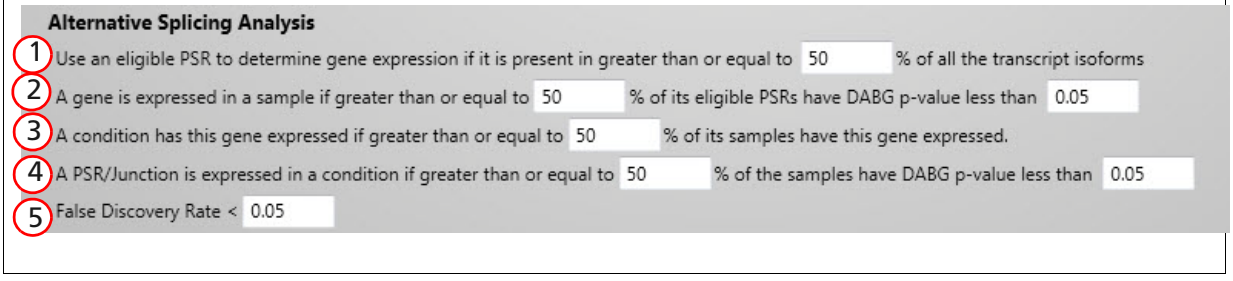


Alternate Splicing Analysis

Here you can customize algorithm parameters by typing values in the text boxes (Figure 1.12).

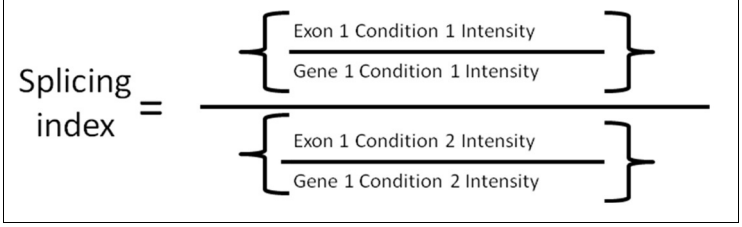
NOTE: Alternate Splicing Analysis is only available for certain arrays. Please contact Affymetrix support regarding which array is supported.

Figure 1.12 Configurable Parameters 1-5



The Splicing Index algorithm compares normalized signal estimates from one condition to another. See the equation below: (Figure 1.13)

Figure 1.13 Splicing Index Algorithm

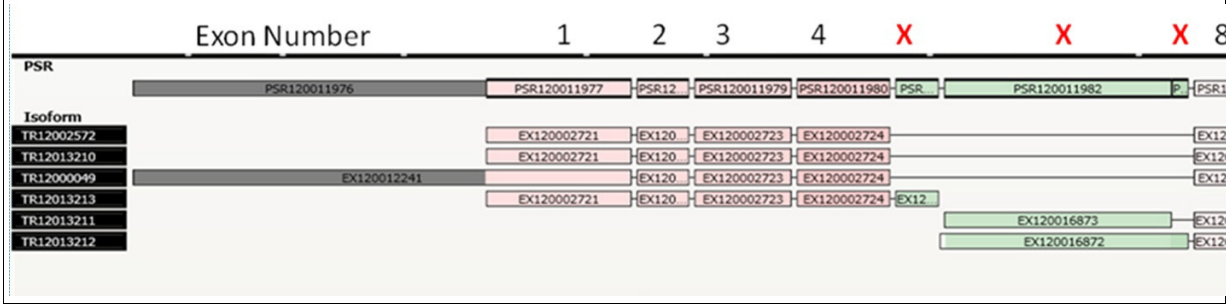


In order for Transcriptome Analysis Console to perform a Splicing Index, two key criteria must be met. They are as follows:

1. **Criteria 1:** A Transcript Cluster gene must be expressed in both conditions. Therefore, for each condition, you need to determine whether a gene is expressed or not.

- Configurable Parameter **3**: A gene can be considered expressed in a condition (if it meets the criteria specified in parameter 3. As an example, if at least 50% of the samples are expressed, then this gene is expressed in this condition).
- Configurable Parameter **2**: You must determine whether a gene is expressed by looking at the DABG p-values for all the eligible exons (PSRs). As an example, at least 50% of eligible PSRs must be expressed (DABG $p < 0.05$) for the gene to be considered expressed.
- Configurable Parameter **1**: The way to decide whether a PSR is eligible, is to see if it presents at least 50% of all the transcript isoforms for that gene. Only PSRs 1, 2, 3, 4, 8 are considered as eligible PSRs in this gene, as shown in the example below. (Figure 1.14)

Figure 1.14 Configurable Parameters



2. **Criteria 2:** A PSR or Junction can only be analyzed by Splicing Index if it expresses in at least one condition

- Configurable Parameter **4**: To decide whether a PSR or junction is expressed in a condition, you need (as an example) to check the DABG p-values (from all samples in that condition) to see whether>=50% of samples have DABG $p < 0.05$.
- After a gene and PSRs/junctions meet the criteria above - normalization and comparison can begin.
- In order to perform normalization, gene intensity needs to be calculated. For a particular gene, the gene intensity for each sample is calculated using the Tukey's Biweight average for all the eligible exons (PSRs) intensities in that gene.
- Next, normalize each PSR or junction intensity using the gene intensity of that sample. (Figure 1.13)
- Normalized intensities from Condition1 is compared to normalized intensities from condition 2 using One-way Between-Subject ANOVA for the PSRs and junctions within a gene.

- Configurable Parameter **5**:After running ANOVA, multi-testing correction is performed using Benjamini-Hochberg Step-Up FDR-controlling procedure for all the expressed genes and expressed PSRs/Junctions (expressed in at least one condition).

NOTE: By default, the Alpha level is set as 0.05 in Parameter 5 (False Discovery Rate field).

Warnings

If algorithm parameters have been changed to other values than default, **red** warning text appears at the bottom of the Preferences window. Below are two warning examples: (Figure 1.15)

Figure 1.15 Warnings

1. A blank/non-number entry generates a ***is not a number!*** warning.
2. Parameter(s) entered, that are different from the default generate a ***Parameter(s) are different than the defaults!*** warning.

NOTE: Warnings are not analysis specific. Example: If you change parameters for a splicing index, then run a gene level analysis, any change warnings remain on screen (regardless if your parameter setting applies to your analysis or not).

Default Button

Click **Default** (bottom right) to RESET Algorithm parameters to their factory settings.

Open Existing Result Tab

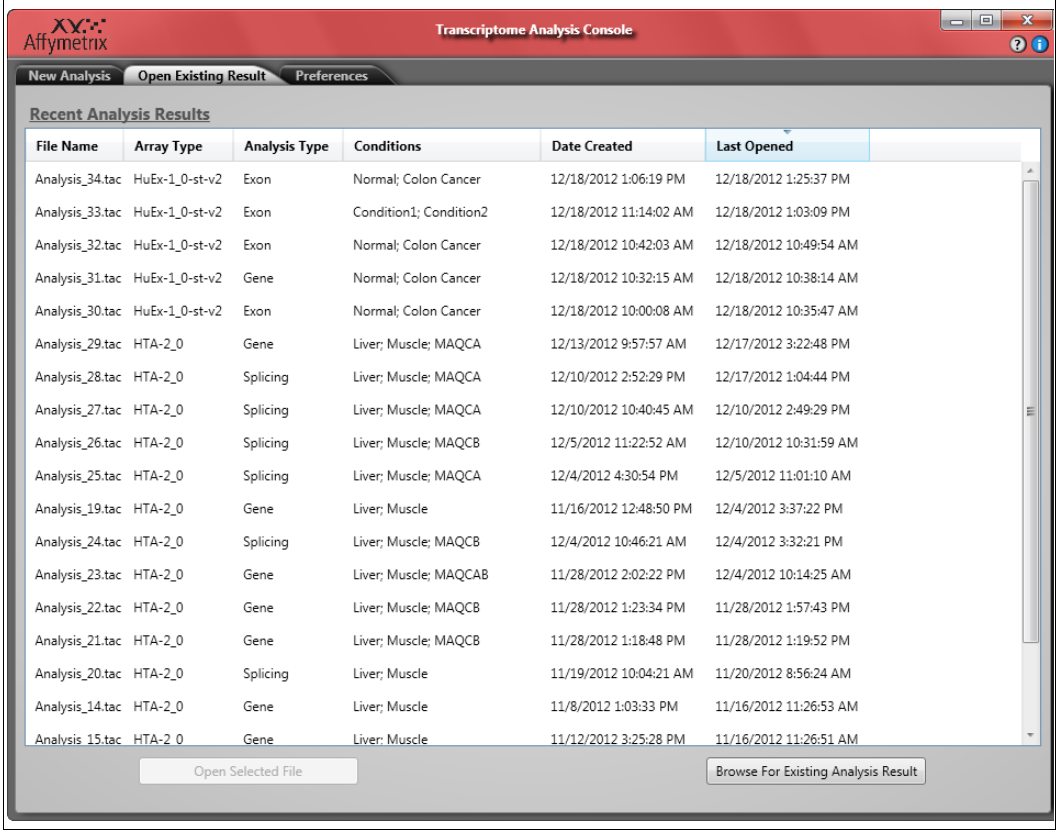
TAC always auto-saves your studies. At any time, you can view recent analysis results.

1. Click on the **Open Existing Result** tab.

TIP: Click any of the window's header columns to sort your recent studies by Ascending (A-Z) or Sort By Descending (Z-A).

The Open Existing Result window appears. (Figure 1.16)

Figure 1.16 Open Existing Result window



2. Double-click on a recent study or single-click on it, then click Open Selected File.

After a few moments, your recent study opens (in the same state - you last left it).

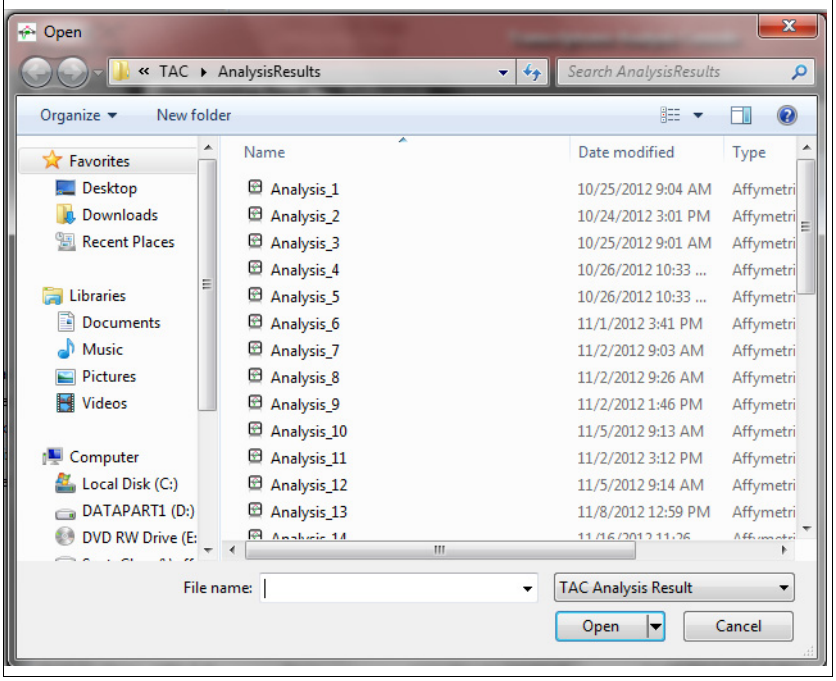
Browsing For Existing Analysis Result

Do the following if you cannot locate a study:

1. TAC stores twenty recent results in the Open Existing Result window. If you still cannot locate your study, click Browse For Existing Analysis Result.

The following window appears: (Figure 1.17)

Figure 1.17 TAC Analysis Results window



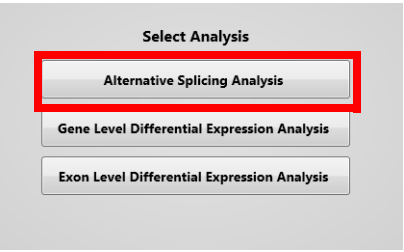
2. Click on a recent study from the TAC Analysis Results window, then click **Open**. After a few moments, your recent study opens (in the same state - you last left it).

Alternative Splicing Analysis

Setting Up an Analysis Using Alt Splice CHP Files

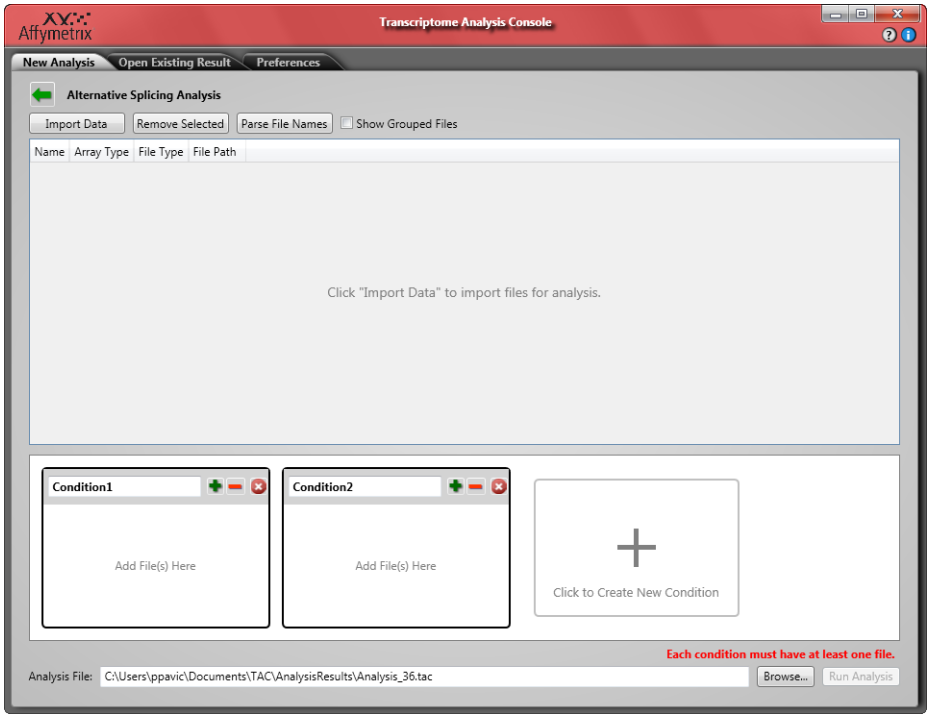
1. At the main TAC window, click **Alternate Splicing Analysis**. (Figure 2.1)

Figure 2.1 Main TAC window



The New Analysis window appears. (Figure 2.2)

Figure 2.2 New Analysis window



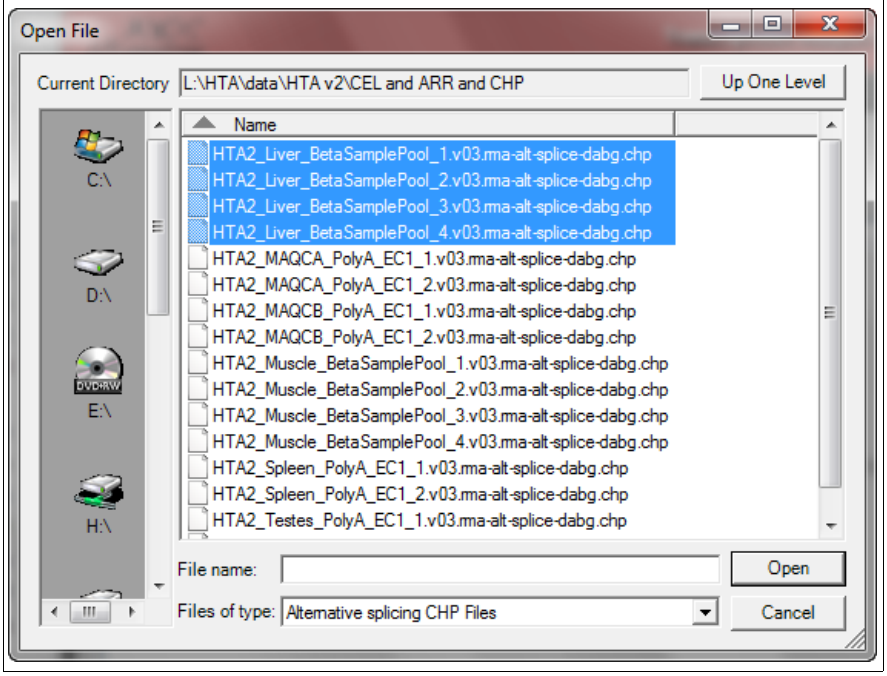
2. Click **Import Data**.

The following window appears. (Figure 2.3) It displays the data path you set up earlier and its files.

NOTE: The first time you launch TAC, it asks you to define a path to store your library and annotation files. For your convenience, TAC retains this path information. Affymetrix recommends you use the Expression Console library path you already configured.

IMPORTANT: To perform an alternative splicing analysis, you must import "alt-splice" .chp files. (Figure 2.3)

Figure 2.3 Import Data Dialog window



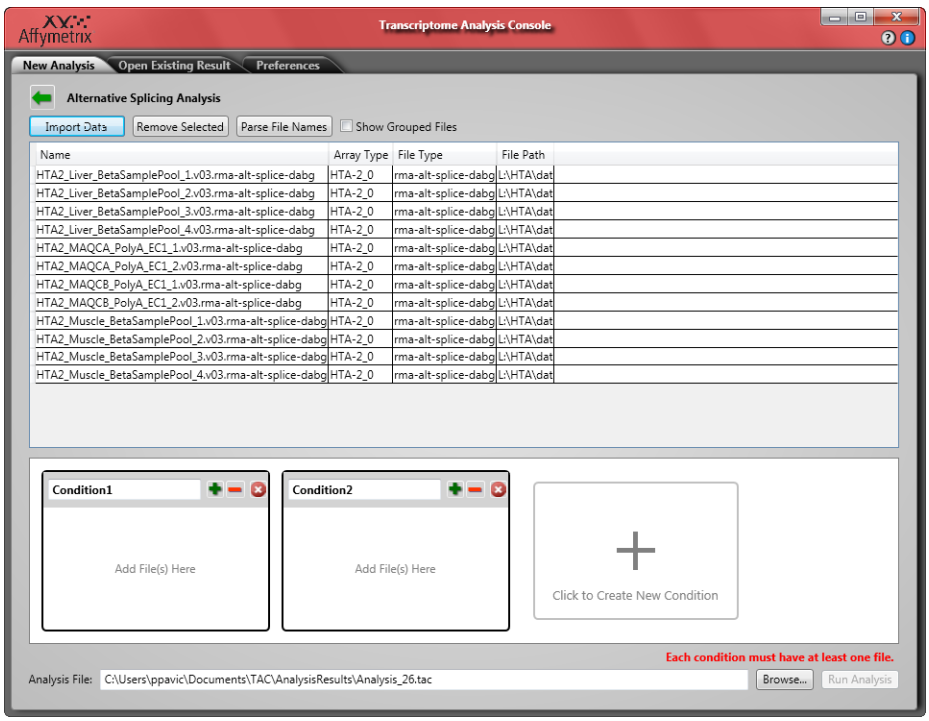
3. Single click, Ctrl click, or Shift click to select multiple files (Figure 2.3).

NOTE: To optimize the analysis, Affymetrix recommends importing more than 1 sample per condition.

4. Click Open.

The selected files are now populated in the Sample File Window. (Figure 2.4)

Figure 2.4 Import Data into Sample File Window



- Click Show Grouped Files checkbox (Figure 2.4) to display sample file names and attributes (even after they have been added to conditions). Samples that have been added to different conditions are displayed in gray.
- Click Remove Selected (Figure 2.4) to remove a file(s) from the Sample File window.

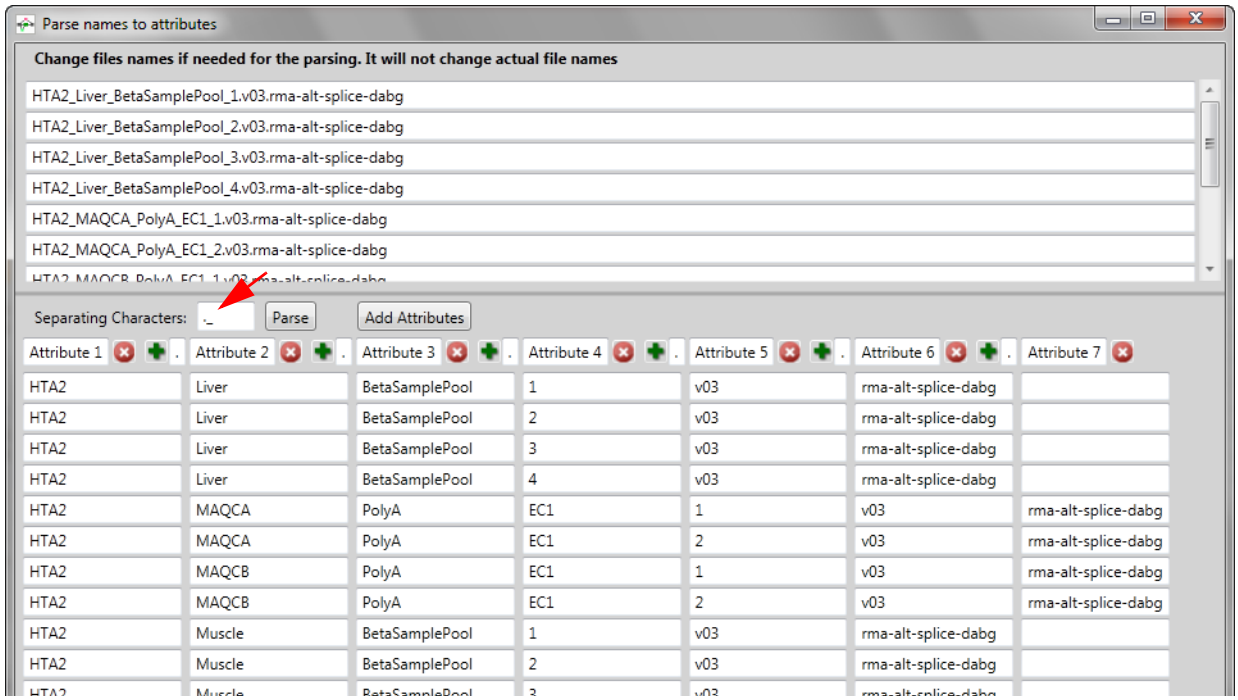
Parsing Imported Data File Names (Optional)

This option gives you the ability to parse attributes from the sample file names and helps you set up conditions.

1. Click Parse File Names.

The following window appears: (Figure 2.5)

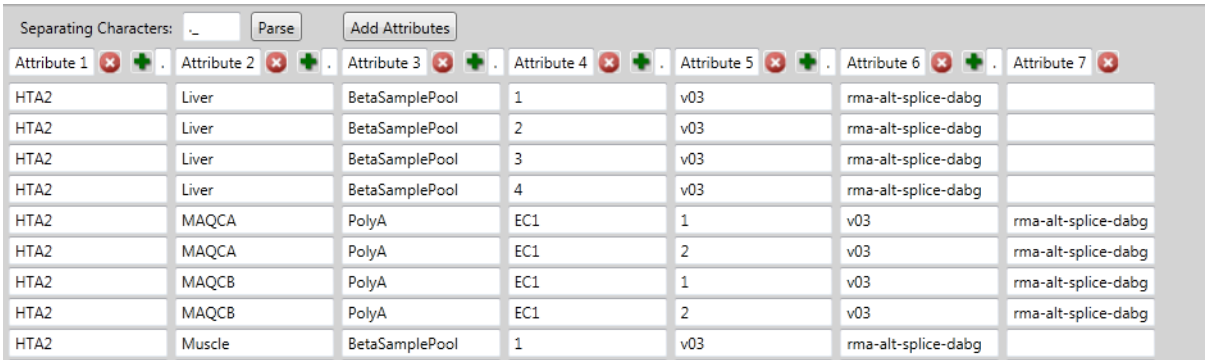
Figure 2.5 Parse File Name window



2. Determine what common separating characters reside within your file names. The file name examples (in Figure 2.5) are separated by an underscore and period.
3. Type the appropriate symbols in the **Separating Characters** field. In this example, an underscore and period.
4. Click **Parse**.

The Parse File Names table now appears as shown. (Figure 2.6)

Figure 2.6 Parse File Name table

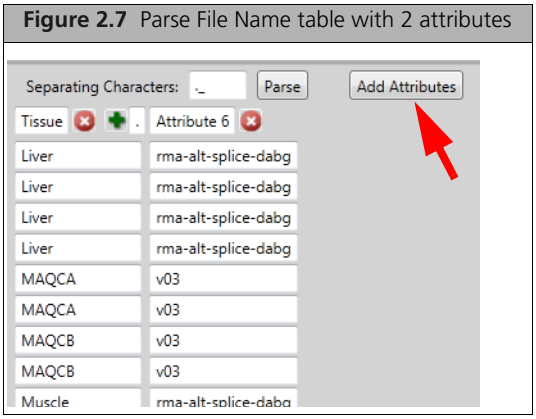


5. Do the following to clean up attributes parsed from the sample file names:

- Click inside any of the Attribute text fields Attribute 1 to type in a new Attribute name.
- If desired, click . to change the default separating character for combing your parsed attributes. The default separating character is a period. These separating characters are useful if you ever want to combine multiple parsed attributes to a new attribute.
- Click to join together a neighboring attribute column.
- Click to remove an attribute column from the table.

In the example above, since Attribute 1, 3, 4, 5, and 7 are redundant and not useful, they are removed. The table now appears as shown. (Figure 2.7)

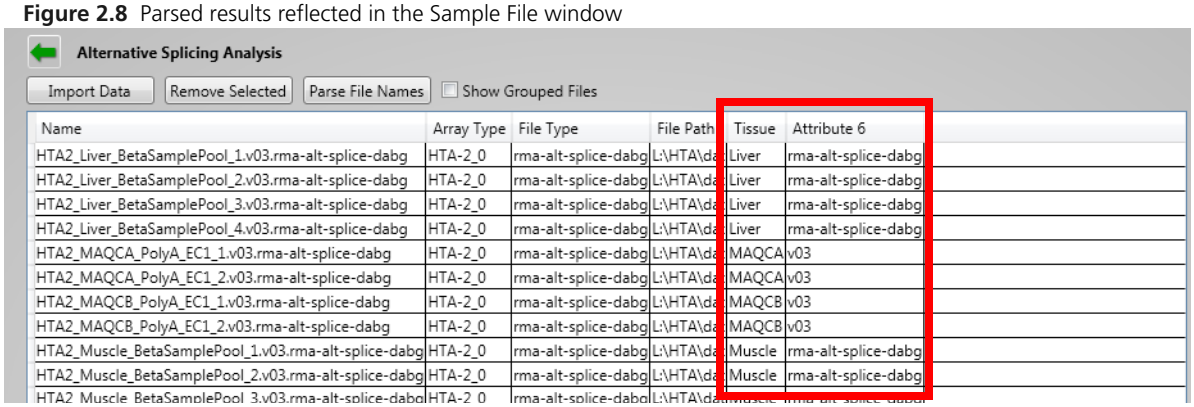
Figure 2.7 Parse File Name table with 2 attributes



6. To save your parsed attributes to the Same File window, click Add Attributes.

The parsed file name attributes (Figure 2.7) are added to the Sample File window as additional attributes. (Figure 2.8)

Figure 2.8 Parsed results reflected in the Sample File window



Importing CHP Files into Different Condition Groups

IMPORTANT: Customize your condition names first, then add the CHP files into each condition.

1. Click on the **Condition1** window header field to rename it to an appropriate Condition name. (Figure 2.9)
2. Click to select and highlight the data you want to use for Condition1.
3. Click in the Condition1 window to add your selected files to the Condition1 window.
4. If needed, click to move selected files back to the Sample File window.
5. If needed, click to delete your current Condition and move all its files back to the Sample File window.
6. Repeat the steps 1-3 (above) for Condition2.
7. To create more than 2 conditions, click **Click to Create New Condition** (Figure 2.9), then repeat steps 1-3 (above) for your 3rd Condition.
8. If needed, edit your Analysis result file path and/or name by clicking inside the **Analysis File** text field (Figure 2.10), or click **Browse** to select a new file destination.

Importing Files using Drag and Drop

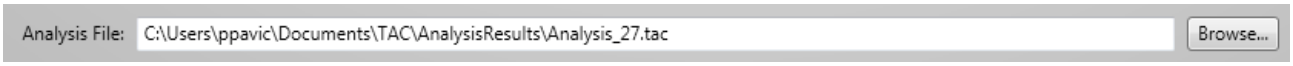
1. Click Shift or click Ctrl, to select a group of files.
2. Click and hold onto the last file in the group, then drag them into the appropriately labeled Condition window.
3. Release the mouse button.

The Condition1 (Liver) window now contains your files.

Figure 2.9 Edited Condition1 name with samples

4. Repeat the steps 1-3 (above) for Condition2.
5. To create more than 2 conditions, click **Click to Create New Condition** (Figure 2.9), then repeat steps 1-3 (above) for your 3rd Condition.
6. If needed, edit your Analysis result file path and/or name by clicking inside the **Analysis File** text field (Figure 2.10), or click **Browse** to select a new file destination.

Figure 2.10 Editable Analysis Result File Paths

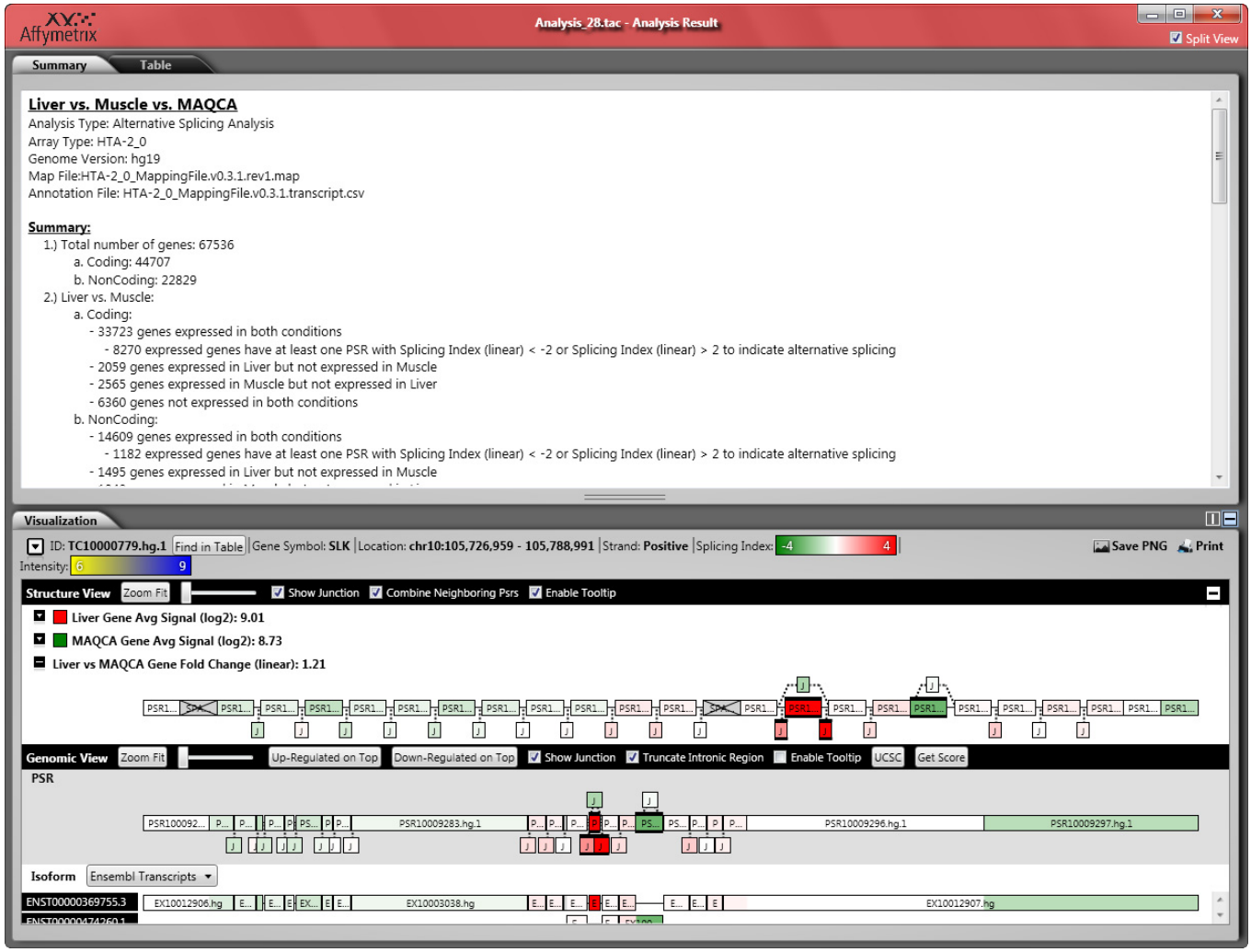


7. After the Conditions have been labeled and populated, click Run Analysis.

! TIP: TAC auto-saves your studies. At any time, click on the Open Existing Result tab to view recent analysis results.

Please Wait... appears. then (by default), the Summary and the Splicing Viewer appear together using a split window view. (Figure 2.11)

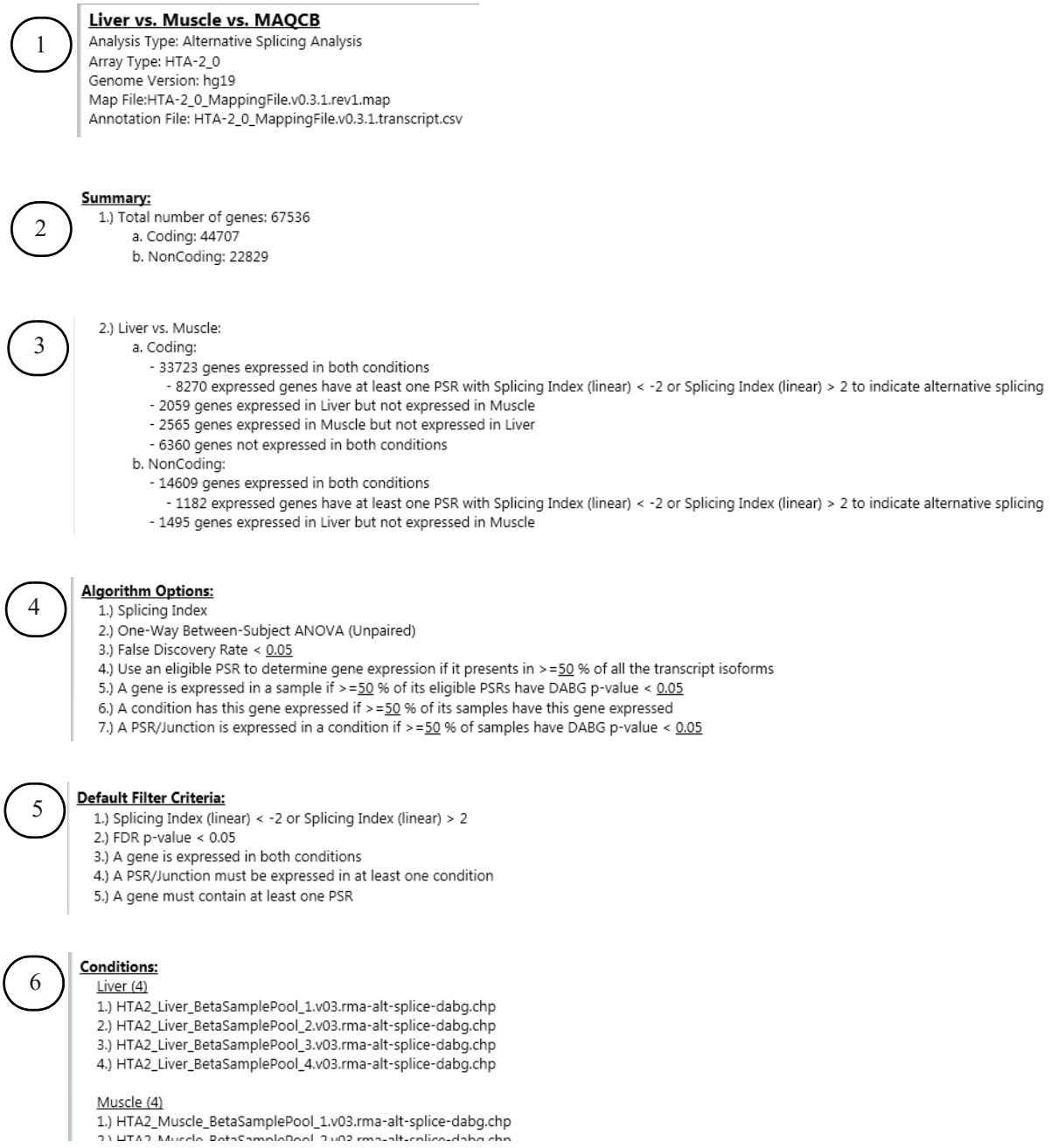
Figure 2.11 Summary and Splicing Viewer



Alternative Splicing Analysis Summary Window (Overview)

The information shown in the summary is based on the algorithm parameters applied during the analysis. See the example below: (Figure 2.12)

Figure 2.12 Summary Window



1. Summary information per this Alternative Splicing Analysis. NOTE: Summaries vary between Gene, Exon, and Splicing analysis.
2. Array summary. Total number of transcript clusters (genes), and numbers of coding and noncoding transcript clusters on this array.
3. Summary of the Coding transcript clusters. In the example above, 33723 transcript clusters passed the Splicing Index criteria of ***Genes need to be expressed in both conditions***. 8270 transcript clusters have at least one Probe Selection Region (PSR) with Splicing Index (linear) < -2 or Splicing Index (linear) > 2. The group of genes listed below (2059, 2565, and 6360) represent the genes that do not meet the Splicing Index criteria of ***Genes need to be expressed in both conditions***.

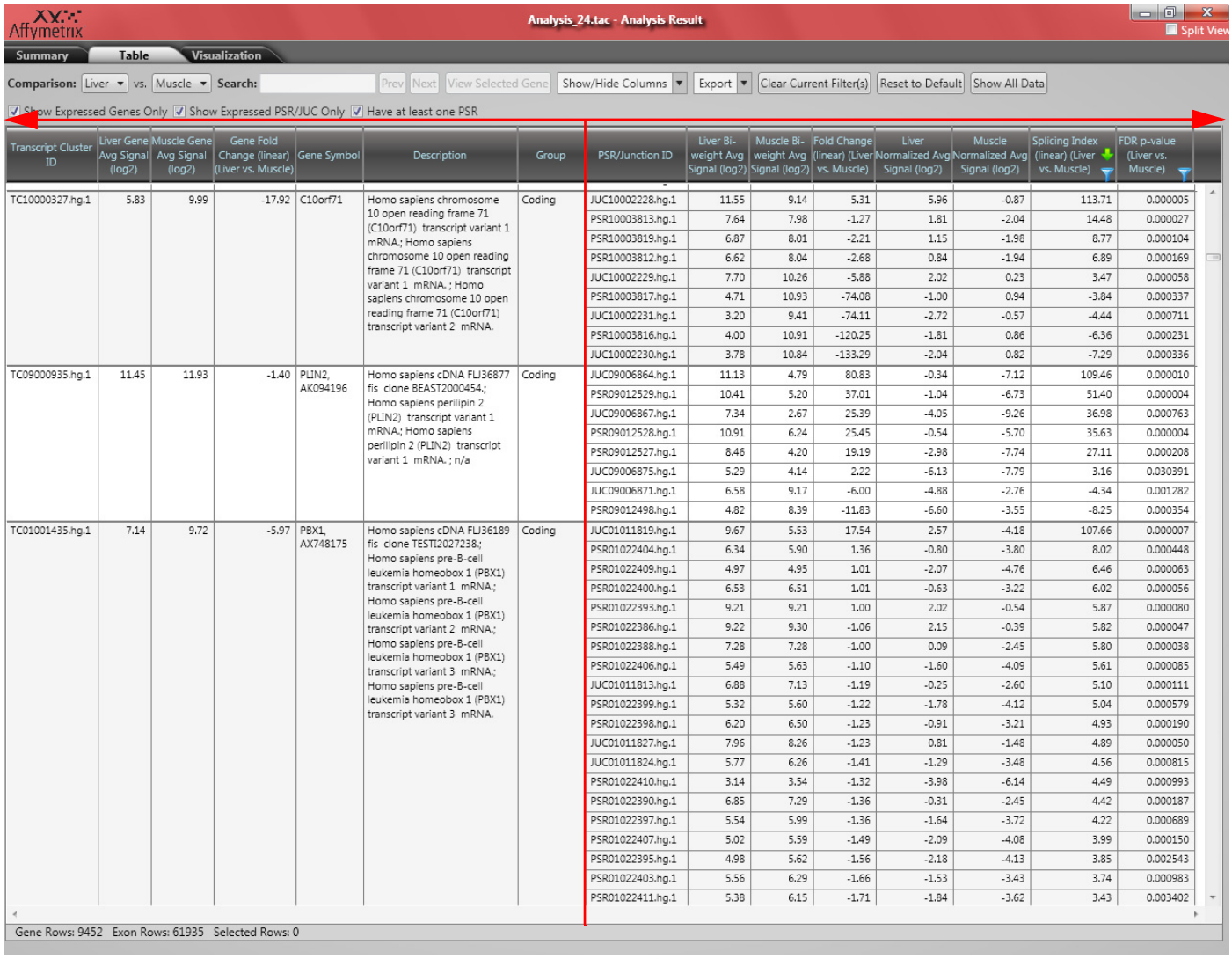
4. Displays the algorithm parameters used to perform the splicing analysis.
5. This section displays the factory default filtering criteria results. NOTE: Only transcript clusters that pass in the Splicing table criteria are summarized.
6. Displays each Condition name and the total number of CHP files in it. Scroll down to reveal the other Conditions in your analysis.

Alternative Splicing Analysis Table Window (Overview)

The table results are based on the algorithm parameters applied during the analysis. The information shown is divided into 2 parts. (Figure 2.13)

- The left side of the table provides gene level information.
- The right side of the table provides PSR/Junction information and is organized by each transcript cluster.

Figure 2.13 Table window



Parts of the Table

- Column Headers
- Table Options

Column Headers

The factory default columns and 2 preset filters are as shown. (Figure 2.14)

See Table 2.1 for definitions of these columns.

Figure 2.14 Default Table Column Headers

Showing or Hiding Table Columns

1. Click the Show/Hide Columns drop-down menu to show or hide columns in the splicing table.
2. Click outside the Show/Hide Drop-down menu to close it.

Table 2.1 Available Splicing Table Columns (BOLD columns represents columns that TAC displays by default)

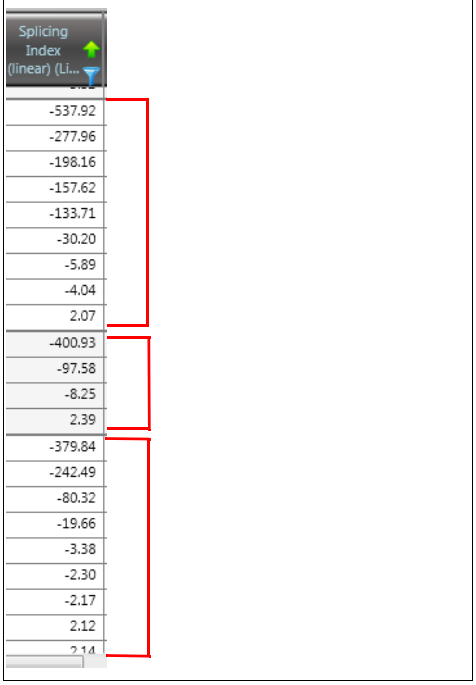
Available Columns	Description
Transcript Cluster ID	ID of Transcript Cluster (TC)
(Condition1) Expressed (Condition2) Expressed	True or False statement regarding whether this TC is expressed in a condition based on criteria specified in the Algorithm Options. A condition has this gene expressed if greater than or equal to 50 % of its samples have this gene expressed. As an example, True means >= 50% samples are expressed in this condition False means < 50% samples are expressed in this condition
(Condition1) Expressed (Detail) (Condition2) Expressed (Detail)	Number of expressed samples/total samples in a condition. A gene is expressed in a sample if greater than or equal to 50 % of its eligible PSRs have DABG p-value less than 0.05 As an example, 4/4 means 4 out of 4 samples met this criteria: >=50% eligible PSRs have DABG < 0.05 1/4 means only 1 out of 4 samples met this criteria: >=50% eligible PSRs have DABG < 0.05
(Condition1) Gene Avg Signal (log2) (Condition2) Gene Avg Signal (log2)	Tukey's Bi-weight[1] average of gene level intensity of all the samples in a condition: Bi-weight average of (sample 1 gene1 intensity + sample 2 gene1 intensity +…+ sample N gene1 intensity)
(Condition1) Standard Deviation (Condition2) Standard Deviation	Standard Deviation[2] of gene intensities from all samples in a condition: STDEV of (sample 1 gene1 intensity + sample 2 gene1 intensity +…+ sample N gene1 intensity)
Gene Fold Change (linear) (Condition1 vs. Condition2)	This shows the fold change (in linear space) of Condition1 vs. Condition2. $2^{[\text{Condition1 Gene Avg Signal (log2) - Condition2 Gene Avg Signal (log2)}]}$
Gene Symbol	Gene symbol for this transcript cluster. Note: RefSeq gene symbol is listed as the first gene symbol (if there are more than 1 gene symbol). Note: A TC with no gene symbol, may be auto-assigned a public gene ID.
Description	Gene Description for this TC.
Chromosome	Chromosome for this transcript cluster. See Chromosome Naming Scheme[3] for a detailed description.
Genomic Position	Genomic Start/Stop position for this TC.
Public Gene IDs	Public Gene IDs for this TC.
Group	Whether this TC is coding, non-coding, or other.
PSR/Junction ID	ID of Probe Selection Region (PSR) and Junction Probe Sets.

Available Columns	Description
Eligible	True or False statement regarding whether this PSR presents in all the transcript isoforms based on criteria specified in the Algorithm Options.  As an example, True - 65.00% means this PSR is eligible for determining gene intensity because it presents in 65% of all the transcript isoforms (when the cutoff is 50%) False - 21.42% means this PSR is not eligible for determining gene intensity because it only presents in 21.42% of all the transcript isoforms (when the cutoff is 50%) Note: If no PSR is eligible (based on the specified criteria), all PSRs are considered as eligible.
(Condition1) PSR/JUC Expressed (Condition2) PSR/JUC Expressed	True or False statement regarding whether this PSR or Junction is expressed in a condition (based on percentage of samples that meets the DABG cutoff specified in the Algorithm Options).  As an example, True means this PSR or Junction met the criteria: >=50% samples has DABG < 0.05 False means this PSR or Junction didn't meet the criteria: >=50% samples has DABG < 0.05. Example: only 25% samples has DABG < 0.05 Note: Only PSR/Junctions that are expressed in at least one condition can be in the table default view.
(Condition1) PSR/JUC Expressed (Detail) (Condition2) PSR/JUC Expressed (Detail)	Number of samples met the DABG cutoff/total samples in a condition As an example, 4/4 means 4 out of 4 samples met the DABG criteria: DABG < 0.05 0/4 means no sample met the DABG criteria: DABG < 0.05
(Condition1) Bi-weight Avg Signal (log2) **(Condition2) Bi-weight Avg Signal (log2)**	Tukey's Bi-weight[1] average of PSR or junction intensity of all the samples in a condition: Bi-weight average of (sample 1 PSR1 intensity + sample 2 PSR1 intensity +…+ sample N PSR1 intensity.
Fold Change (linear) (Condition1 vs. Condition2)	This shows the fold change (in linear space) of Condition1 vs. Condition2. $2^{[\text{Condition1 Bi-weight Avg Signal (log2)} - \text{Condition2 Bi-weight Avg Signal (log2)}]}$
(Condition1) Normalized Avg Signal (log2) **(Condition2) Normalized Avg Signal (log2)**	Tukey's Bi-weight[1] average of normalized PSR or junction intensity of all the samples in this condition: Bi-weight average of (sample 1 PSR1 intensity/sample 1 gene1 intensity + sample 2 PSR1 intensity/sample 2 gene1 intensity +…+ sample N PSR1 intensity/sample N gene1 intensity)
Splicing Index[4] (linear) (Condition1 vs. Condition2)	This shows the normalized fold change (in linear space) of Condition1 vs. Condition2 $2^{[\text{Condition1 Normalized Avg Signal (log2)} - \text{Condition2 Normalized Avg Signal (log2)}]}$
ANOVA p-value (Condition1 vs. Condition2)	One-Way Between-Subject ANOVA p-value (Condition 1 vs. Condition2)[2]
FDR p-value (Condition1 vs. Condition2)	FDR adjusted p-value based on Benjamini-Hochberg Step-Up FDR-controlling Procedure[5] IMPORTANT: Only ANOVA p-values from PSR and Junctions that are expressed in at least one condition and its gene must be expressed in both conditions in order to be sent to FDR for correction.

Sorting Columns

TAC uses a 2-level sorting process for the PSR/junction information (right side of the splicing table). First, it sorts PSRs/junctions within each TC based on the PSR/Junction data, then it sorts each gene. For the gene level information (left side of the splicing table), it performs 1-level sort (PSRs/Junctions are auto-sorted by PSR/Junction ID column). (Figure 2.15)

Figure 2.15 2-Level Sort Results (Example)

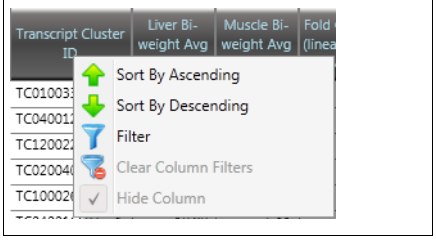


Right-Click Method

1. Select a column, then right-click on it.

The following window appears: (Figure 2.16)

Figure 2.16 Right-click Column Menu



2. Click to select either **Sort By Ascending** (A-Z) or **Sort By Descending** (Z-A).

Double-Click Method

1. Double-click on a column header to sort its data in an ascending order. Double-click on the same column header to sort its data in a descending order.

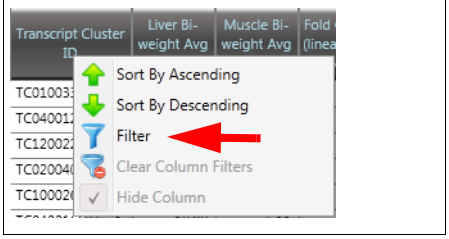
Filtering Column Data

All table columns are filterable.

1. Select a column, then right-click on it.

The following window appears: (Figure 2.16)

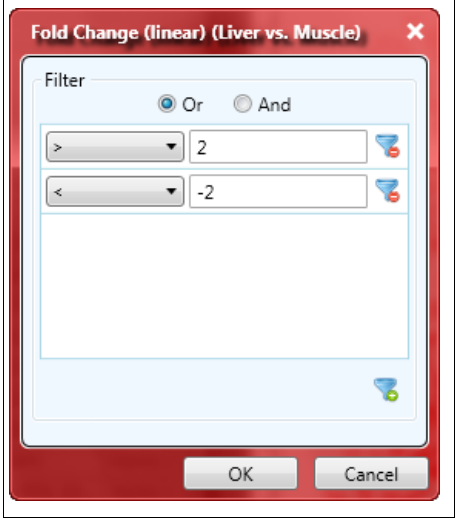
Figure 2.17 Right-click Column Menu



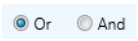
2. Click **Filter**.

The following window appears (Fold Change column example shown): (Figure 2.18)

Figure 2.18 Filter Properties

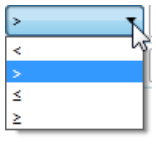


Editing Filtering Properties:

1. Click the **Or** or **And** button to choose **Or** or **AND** logic. 

2. Click the symbol drop-down menu(s) to select new symbol(s).(Figure 2.19)

Figure 2.19 Drop-down Menu



3. Click inside the numbering field(s) 

to enter new value(s).
4. Click  to add filter(s).
5. Click to remove filter(s).

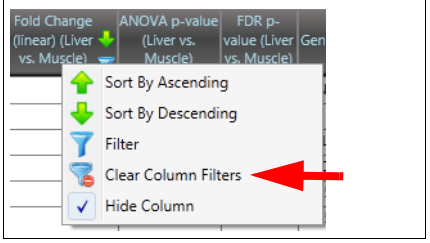
Clearing Filters

Individual Filter

1. Right-click on the filtered column you want to clear.

The following window appears: (Figure 2.20)

Figure 2.20 Right-click Column Menu



2. Click **Clear Column Filters**.

The filter is removed.

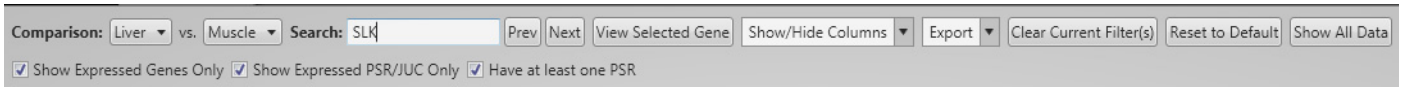
All Filters

1. Click Clear Current Filter(s) remove **ALL** filters from the table headers.

Table Options

Use the Table Options Menu (Figure 2.21) to customize your table view.

Figure 2.21 Table options menu



Show Data Options

By default, all 3 Show Data options are checked. (Figure 2.22) As these options are unchecked, the gene count changes and the summary (below the splicing table) automatically updates the counts.

Figure 2.22 Show Data Options

Show Expressed Genes Only

- Check this box to show ONLY the genes that are expressed in both selected Conditions.
- Uncheck this box to show genes that are NOT expressed in both of your Conditions. Transcript clusters that are expressed in only 1 condition (**False/True, True/False**), or not expressed in either condition (**False/False**) are highlighted in ***italic red***.
- All currently set filters are cleared.

Show Expressed PSR/JUC Only

- Check this box to show PSRs and Junctions that are expressed in at least one of your set Conditions.
- Uncheck this box to show PSRs and Junctions that are NOT expressed in both of your Conditions. The PSR and Junctions that are not expressed in both conditions (False/False) are highlighted in ***italic red***.

- All currently set filters are cleared.

Have at Least One PSR

- Check this box to show genes that have at least 1 PSR that passed your current filter criteria.
- Uncheck this box to show genes that have at least one PSR/JUC that passed the current filter criteria.

Showing All Data

- Click the Show All Data button (top right) to show all data, including the data that did not pass the default Splicing Index algorithm criteria and default table filter criteria. A warning appears advising you that all current filters will be cleared. (Figure 2.23). Click **Yes** to proceed.

Figure 2.23 Show All Data Warning

NOTE: At any time, click Reset to Default to auto-check all 3 data options.

Rearranging Column Orders

1. Click on a column you want to move.
2. Drag it (left or right) to its new location.
3. Release the mouse button.

The column is now in its new position.

Resetting Table Defaults

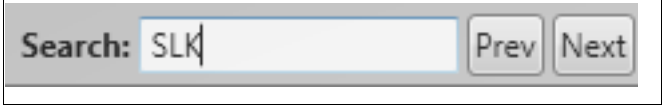
1. Click Reset to Default to return the table to its factory setting.

Searching Keywords

NOTE: The Search Tool is limited to finding matching strings. It is not a full search engine.

1. To search for a keyword within your table, click inside the **Search** field, then type your keyword.
2. Click the Prev or Next buttons to search. (Figure 2.24)

Figure 2.24 Search Tool

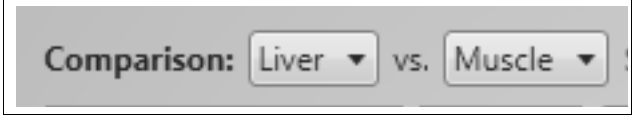


Changing Condition vs. Condition Pairings

1. Use the **Comparison** drop-down menus to change your condition pairings. (Figure 2.25)

You **must** choose 2 different conditions. Identical condition pairings generates the error message, ***Please Choose Two Different Conditions***

Figure 2.25 Condition Pairing Drop-down



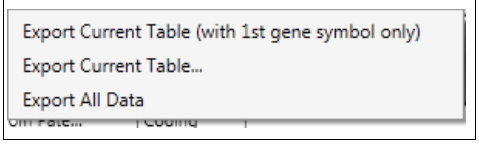
IMPORTANT: Table and graph results ONLY reflect your current Condition pairing.

Exporting Options

If you want to export (Save) your analysis table, click Export drop-down.

The following Export options appear: (Figure 2.26)

Figure 2.26 Export Menu



Exporting the Current Table with 1st Gene Symbols

NOTE: This option shows the first gene symbol only if there is more than one gene symbol for the selected transcript cluster.

1. Click **Export Current Table (with 1st gene symbol only)**.

The **Save As** window appears.

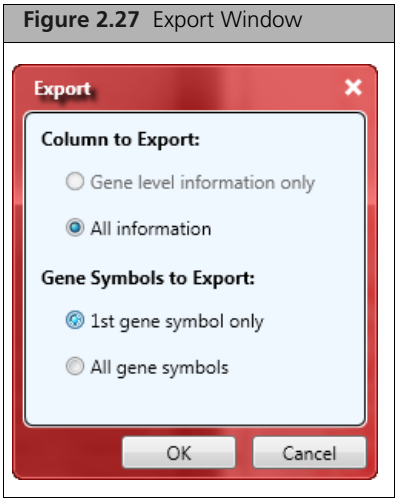
2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting the Current Table

1. Click **Export Current Table**.

The following window appears: (Figure 2.27)

Figure 2.27 Export Window



Column to Export

1. Click either **Gene level information only** or **All information**.

Gene Symbols to Export

1. Click either **1st gene symbol** or **All gene symbols**
2. Click **OK**

The **Save As** window appears.

3. Click on an existing folder or click **New Folder** to choose a new save location.
4. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting All Data

1. Click **Export All Data**.

! NOTE: Only currently paired data is exported, including data in the hidden columns, and the paired data's gene level information.

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Saving Table Information

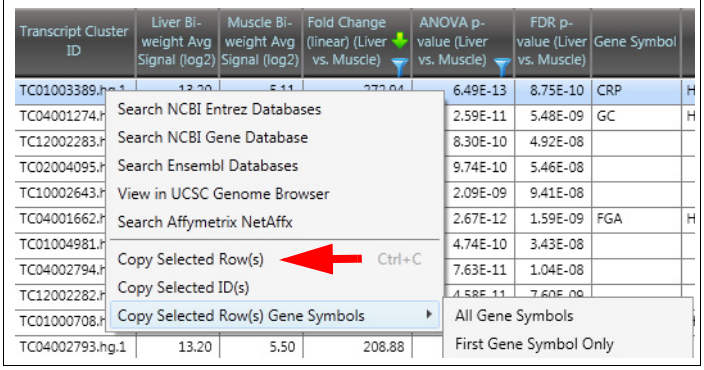
Use this copy feature to save table information to the Windows Clipboard, then use this buffered information for pasting into other applications or websites.

Copy Selected Row(s)

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.

2. Right-click, then click to select **Copy Selected Row(s)**. (Figure 2.28)

Figure 2.28 Copy Selected Row(s) option

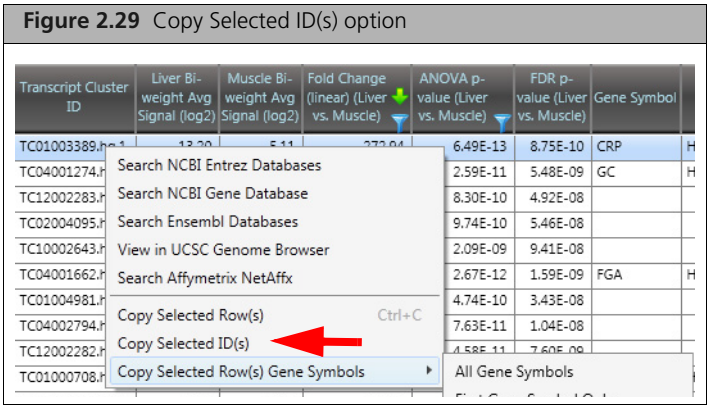


The selected gene level information (shown on the left side of the splicing table) are now copied to the Windows Clipboard for pasting.

Copy Selected ID(s)

1. Click to highlight (light blue) a ID or Ctrl left-click to highlight multiple rows.
2. Right-click, then click **Copy Selected ID(s)** to copy Transcript Cluster IDs). (Figure 2.29)

Figure 2.29 Copy Selected ID(s) option



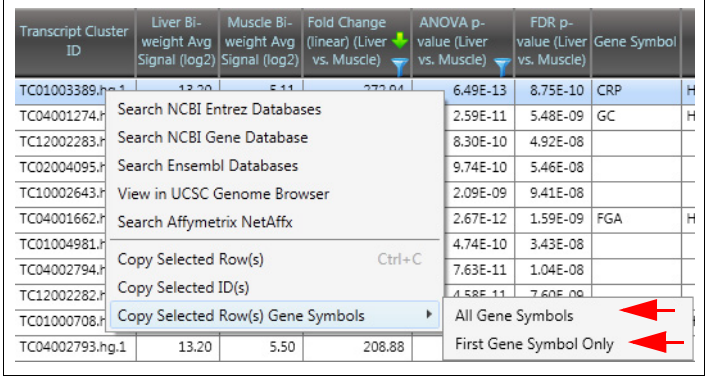
The selected TC IDs are now copied to the Windows Clipboard for pasting.

Copy Selected Row(s) Gene Symbols

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.
2. Right-click on the selection, then click to select **Copy Selected Row(s) Gene Symbols**.

3. Click to select either **All Gene Symbols** (all possible gene symbols for a Transcript Cluster) or **First Gene Symbol Only** (the first gene symbol that belongs to the Transcript Cluster). (Figure 2.30)

Figure 2.30 Copy Selected Row(s) gene Symbols options

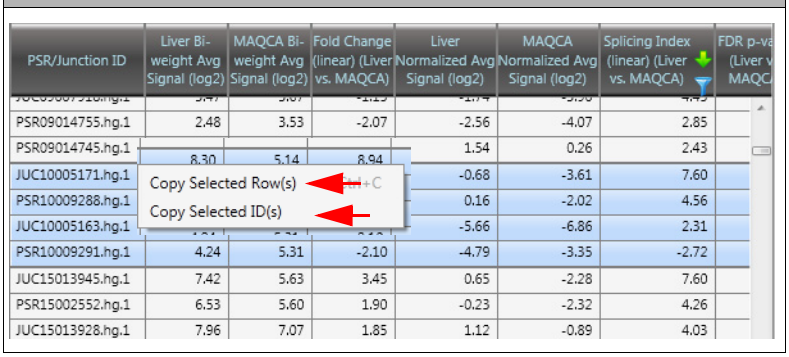


Your selected rows with gene symbols are now copied to the Windows Clipboard.

Copy PSR/Junction Information

1. Click to highlight (light blue) a PSR/Junction ID or Ctrl left-click to highlight multiple rows.
2. Right-click, then click either **Copy Selected ID(s)** or **Copy Selected Row(s)** to copy PSR/Junction IDs. (Figure 2.31)

Figure 2.31 Copy Selected PSR/JUC option



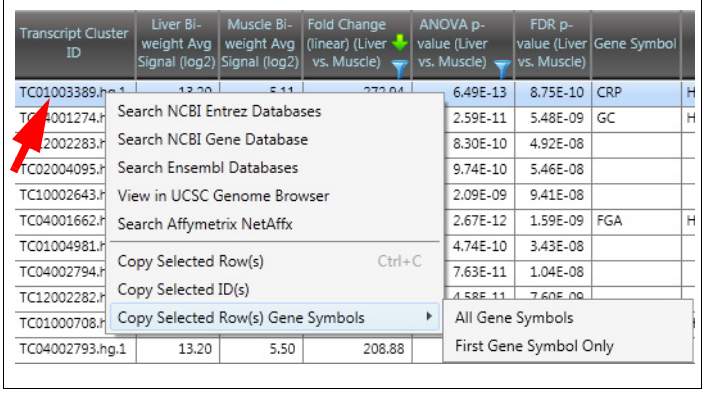
The selected PSR/Junction IDs are now copied to the Windows Clipboard for pasting.

Accessing External Databases (Internet Connection Required)

1. To link out to various external databases, right-click on a TC of interest.

The following menu appears: (Figure 2.32

Figure 2.32 Search Database menu



2. Click to select the external database you want to visit.

Your internet browser opens to the appropriate website.

Searching the Affymetrix NetAffx Website

1. Click to select **Search Affymetrix NetAffx**.

The internet browser opens to the Affymetrix Customer Login window.

2. Enter your NetAffx **Email ID** and **Password**, then click **Submit**.

Your internet browser opens to the ***Netaffx Query Center*** and displays information about your gene of interest.

NOTE: The NetAffx Query Center is compatible with Windows Internet Explorer and Firefox. Chrome is not supported at this time.

NOTE: If a Probe Set or Transcript Cluster is not available, an appropriate message appears.

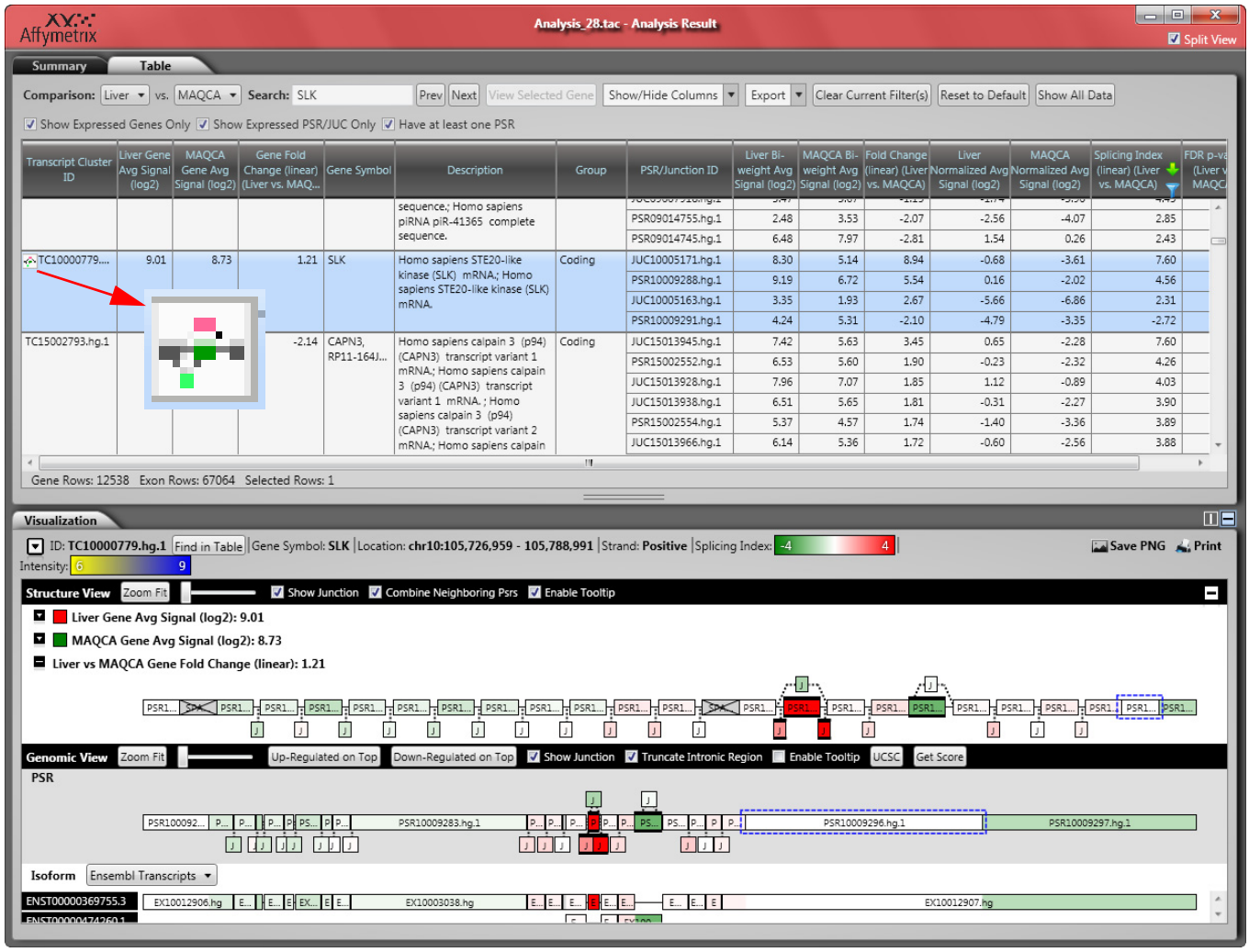
Viewing Results in the Splicing Viewer

NOTE: The Splicing Viewer (lower panel) only reflects the active TC in the table (the TC with the TAC icon next to it). (Figure 2.33)

Using the Splicing Table and Splicing Viewer

1. Double-click on any TC (row), or single-click on it, then click View Selected Gene to see the results in the Splicing Viewer.

Figure 2.33 Splicing Table and Viewer



Visualization Tab

Parts of the Visualization Tab

Figure 2.34 Information header

- Information about the currently selected (active) TC.
- Find in Table button.
- Splicing Index scale.
- Intensity scale.
- Save as PNG.
- Print
- Vertical and Horizontal Split View buttons.

Changing the Factory Set Scale Limits

1. Click  to expand the information header and show the Splicing Index and Intensity scales.

Figure 2.35 Expanded Information Header

Setting New Scale Ranges

1. Click to check the **Auto Scale** checkbox to use the minimum (lower bound) and maximum (upper bound) splicing index values/intensities as the min/max scale for display. (Figure 2.35)
2. Click to uncheck the Auto Scale checkbox to set a fixed scale, then enter your min and max number scales. This newly fixed scale is now saved for use with other TCs and genes.

! NOTE: For the Splicing Index scale the *lower bound* cannot be higher than -1. The *upper bound* cannot be lower than 1.

Changing Scale Colors

1. Use the drop-down color menus to change scale color properties. (Figure 2.35)

Find in Table

1. Click Find in Table to restore the current displayed TC (in the splicing viewer) back to the current table view.

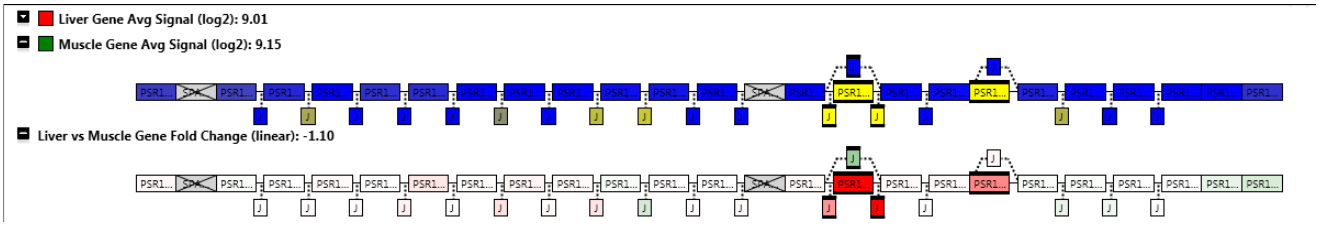
- Click  to return the Information Header to its collapsed view.
- Click either  to view the Splicing Table and Splicing Viewer side by side or top and bottom.

Structure View

- Structure View displays gene structure. All PSRs and Junctions are represented in the structure view with boxes that have same size.
- An Inclusion junction detects 2 neighboring PSRs. The PSRs detected by an inclusion junction are linked and graphically represented as dotted lines when you mouse over or click that junction.
- An Exclusion junction detects PSRs that are apart from each other. The PSRs detected by an exclusion junction are linked and graphically represented as dotted lines when you mouse over or click that junction.
- A crossed-out box represents a PSR/Junction that does not contain data.
- A diagonally-crossed-out box represents a PSR/Junction that is not expressed in at least one condition.
- PSR1... (bold top and bottom borders) represents a PSR/Junction that has passed through the current table's filtering criteria. This only applies to PSRs/Junctions currently filtered in the table.
- A **Spacer** represents a transcript cluster (TC) probe selection region (PSR) where a selection of probes is not possible. Note: Spacer is typically a region with less than 25 bases. Occasionally, some of them can exceed 25 bases.

Parts of the Structure View

Figure 2.36 Structure View



- Intensity Track 1 or (Condition1) Gene Avg Signal (log2) **[9.01]** (Figure 2.36)
- Intensity Track 2 or (Condition2) Gene Avg Signal (log2) **[9.15]**
- Splicing Index Track or (Condition1 vs. Condition2) Gene Fold Change (linear) **[-1.10]**
- Show Junction checkbox
- Combine Neighboring Psrs checkbox
- Enable Tooltip checkbox
- Zoom Fit button
- Zoom Tool Slider bar
- Save as PNG
- Print

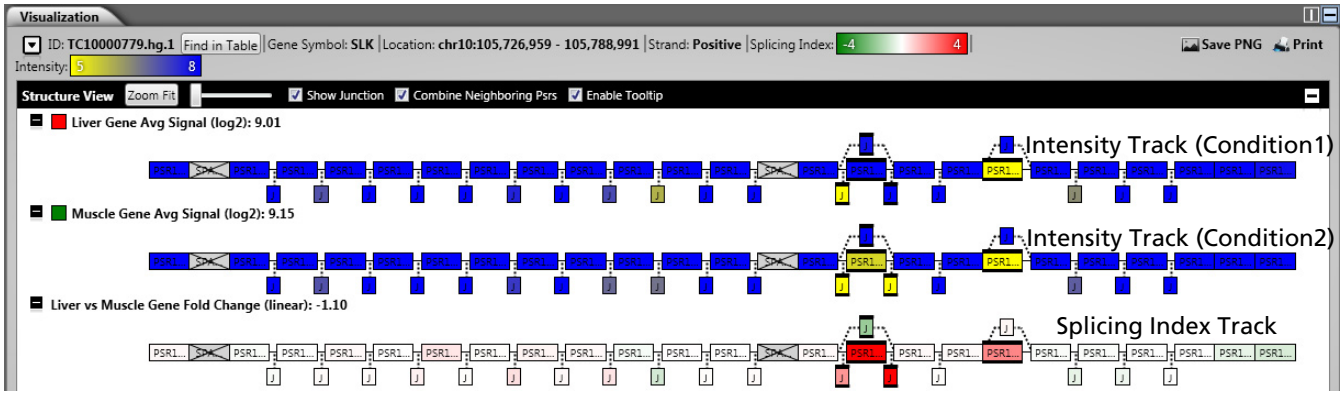
Using the Intensity and Splicing Index Tracks with the Table

The Structure view displays 3 tracks:

- Intensity Track 1 (top) shows the Bi-weight Avg Signal (log2) from each PSR and Junction for Condition1.
- Intensity Track 1 (middle) shows the Bi-weight Avg Signal (log2) from each PSR and Junction for Condition2.
- The Splicing Index Track (bottom) shows the Splicing Index values (linear) from the Condition1 and Condition2 comparison.

1. Click each Intensity Track (far left) to expand (show) them. (Figure 2.37)

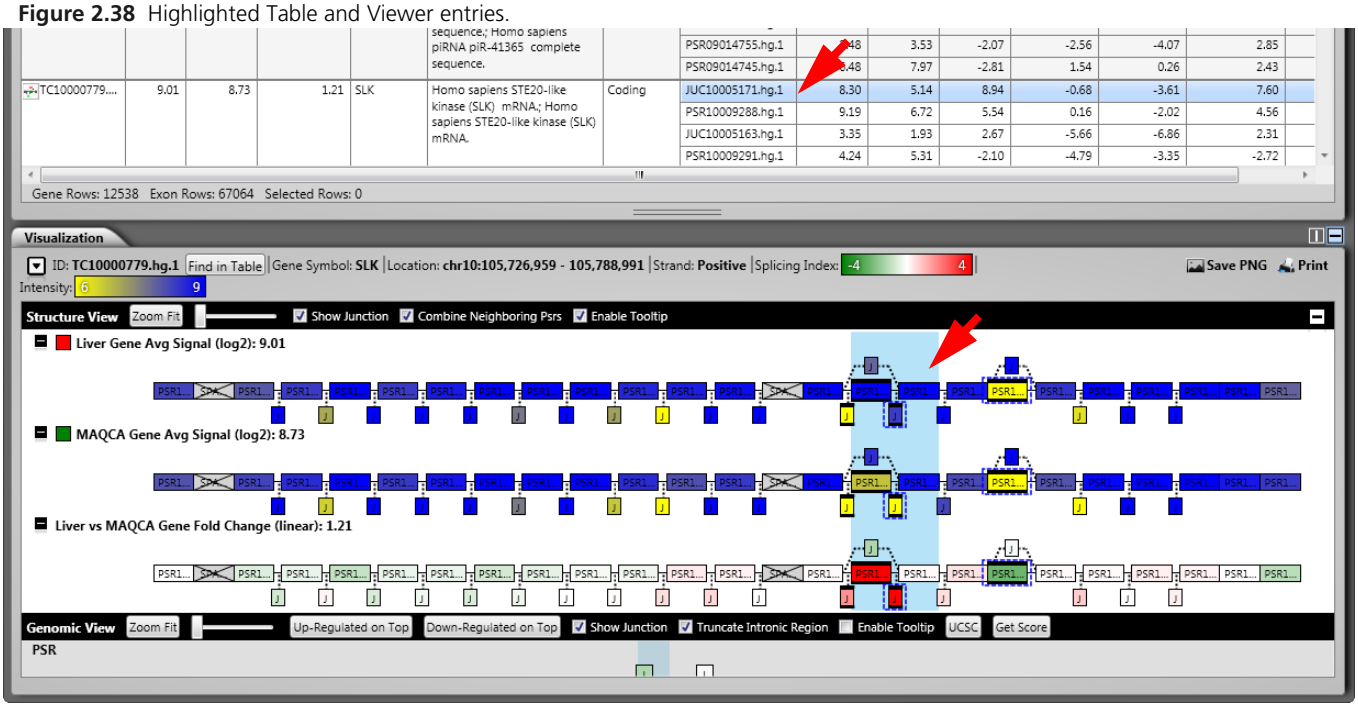
Figure 2.37 Intensity and Splicing Index Tracks



2. From the Splicing Table, click on a PSR/Junction of interest. (Figure 2.38)
3. The selected entry in the splicing table is also highlighted in the Splicing Viewer. (Figure 2.38)

TIP: When you click to select a Junction, its detected PSRs are also highlighted.

Figure 2.38 Highlighted Table and Viewer entries.

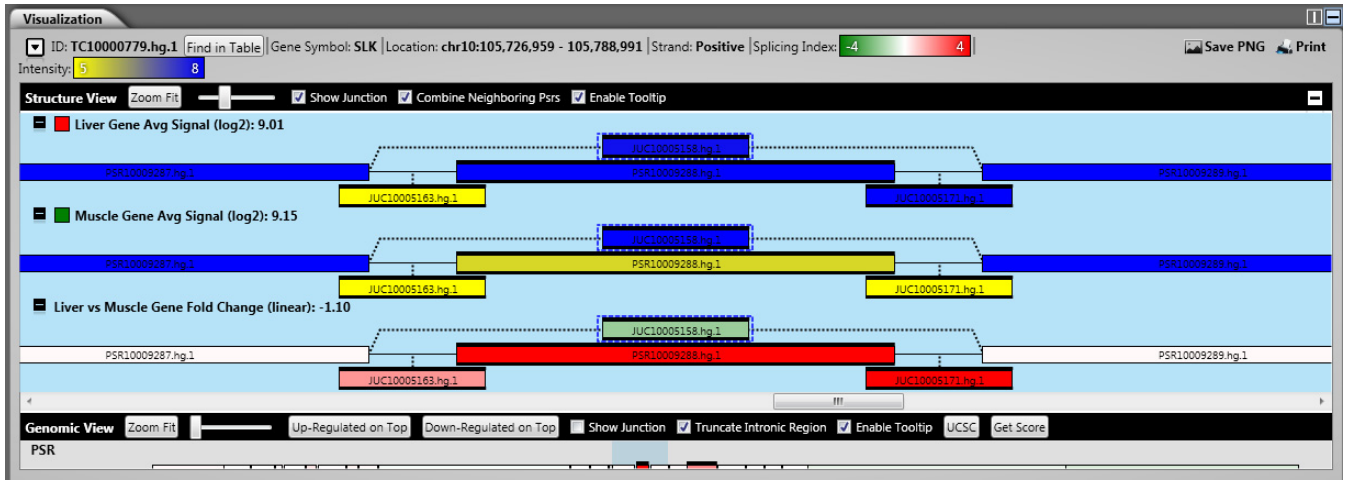


Using the Zoom Feature

1. **Zoom Method 1:** To zoom in on a region of interest, left-click, hold, then move the mouse to frame your selection. Release the mouse button. (Figure 2.39)
2. **Zoom Method 2:** Left-click and hold onto the slider bar, then move it right to magnify your view. Use the horizontal scrolling bar to center your view, as this zoom method is not based on your selection.

3. **Undo:** Click Zoom Fit to return to the default view.

Figure 2.39 Zoom View

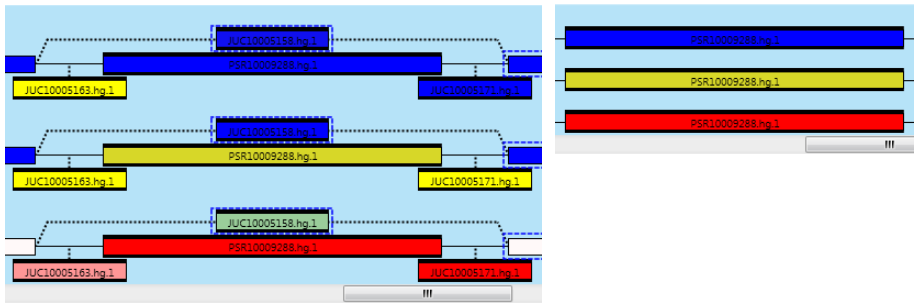


Using the Show Junction Option

The Show Junction option is checked by default and uses dotted lines to show the relationship of the detected Junctions and PSRs.

Uncheck this box to hide these junction lines. (Figure 2.40)

Figure 2.40 Show Junction Option ON and OFF



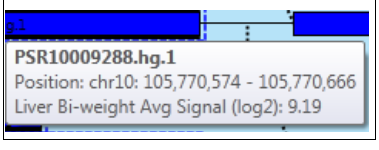
Using the Combine Neighboring Psrs Option

The Combine Neighboring Psrs option is checked by default and link 2 PSRs together (if they are physically right next to each other). Uncheck this box to turn this option off.

Using the Enable Tooltip Option

The Enable Tooltip option is checked by default. Mouse over (position the cursor over) a PSR or Junction to see the details related to that PSR or junction. (Figure 2.41)

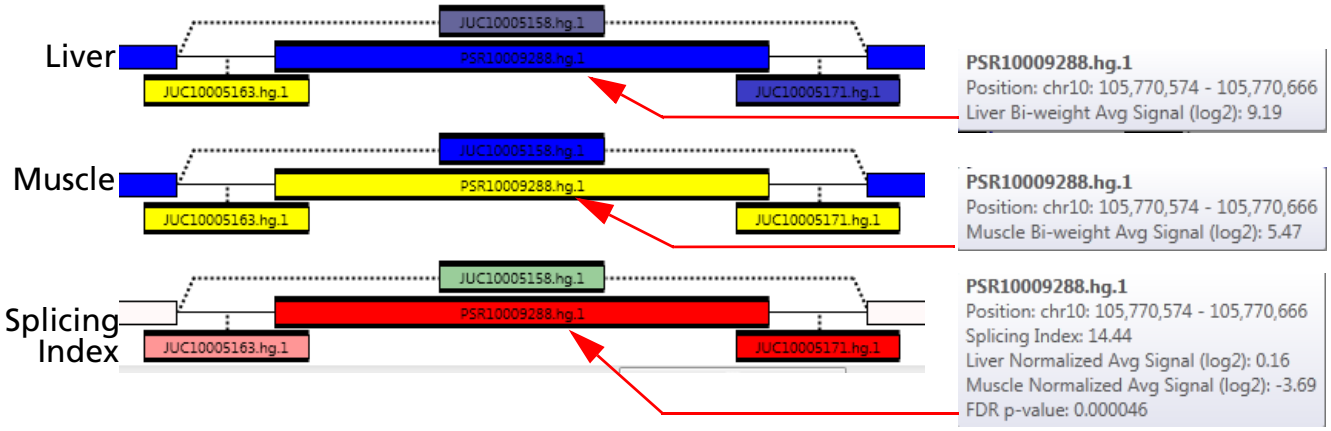
Figure 2.41 Tooltip Example



Identifying an Alternative Splicing Event using Structure View

In the example below (Figure 2.42), the Muscle Bi-weight Avg Signal (log2) of this PSR (PSR10009288) is low (5.47) compared to the Liver Bi-weight Avg Signal. The left (JUC10005163) and right (JUC10005171) inclusion junctions signals are low in Muscle, while the exclusion junction (JUC10005158) signal is high in Muscle. The Splicing Index results show a high SI value for this PSR, high SI values for inclusion junctions, and a low SI value for the exclusion junction. This data indicates this PSR is preferentially included in the Liver, but not in the Muscle. Therefore, this PSR is likely to be an alternative Cassette Exon. There is an Exon Skipping Event in muscle.

Figure 2.42 Alternative Splicing Event Example



Click ⊟ to return the Information Header to its collapsed view.

Genomic View

- The Genomic View displays gene structure and transcript isoforms that belong to a TC.
- All PSRs and Junctions are represented as their true genomic sizes. Example: The larger the box, the larger the exon.
- Each PSR/Junction color is based on the color used in the Splicing Index track.
- Below each gene structure, transcript isoforms are displayed in order. The order starts with the transcript isoforms that most likely exist in Condition1 to transcript isoforms that most likely exist in Condition2.
- The transcript isoforms that fit Condition1 are auto-sorted to the top. Transcript isoforms that fit Condition2 are auto-sorted to the bottom.

How Each Isoform Sorting Score is Calculated

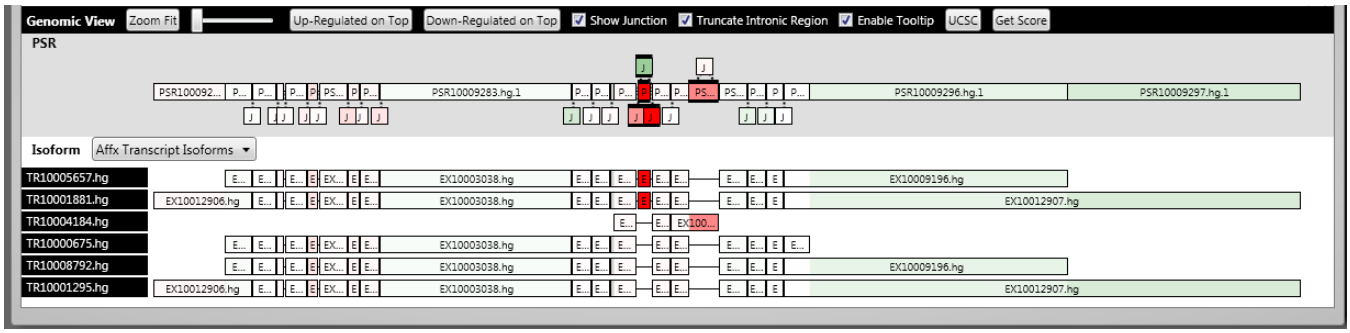
- All Splicing Index equations use log2 scale data.
- TAC sorts each selected PSR. If no PSRs are selected, then all available PSRs are used.
- If the PSR has data and is expressed in at least one of the conditions, then TAC checks whether it is presented in an isoform or not.
- If the isoform has at least one exon that contains a specific PSR, then it is considered Present.

The score for a specific PSR is calculated as follows:

- If FDR **p-value < p value cutoff** (set in Preferences page), then **ScoreMultiplier = 1.5, otherwise the ScoreMultiplier = 1**
- If Up Regulated and present, then score = **Splicing Index * ScoreMultiplier**
- If Up Regulated and not present, then score = **- Splicing Index * ScoreMultiplier**
- If Down Regulated and not present, then score = **- Splicing Index * ScoreMultiplier**
- If Down Regulated and present, then score = **Splicing Index * ScoreMultiplier**
- If Splicing Index = **0, then score = 0**
- Score of the Isoform = the sum of the PSR(s) scores
- If the user selects "Ensembl Transcripts" or "RefSeq Genes", then the software performs 2-level sorting. It sorts Isoforms with IDs first, then it performs a sub-sort within the first sorted result.

Parts of the Genomic View

Figure 2.43 Genomic View Example



- Up Regulated on Top button
- Down Regulated on Top button
- Show Junction checkbox
- Truncate Intronic Region checkbox
- Enable Tooltip checkbox
- UCSC button

- Get Score button
- Isoform drop-down menu

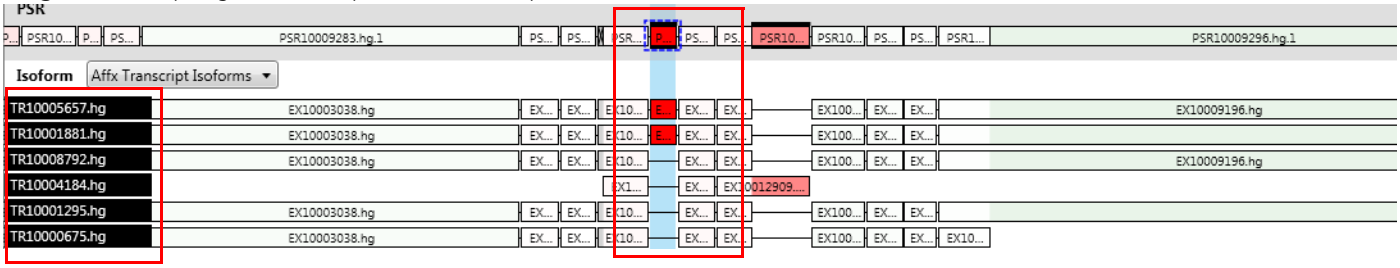
Using the Up and Down Regulated on Top Buttons

1. Click to highlight a PSR, then click Up-Regulated on Top button to bring the isoforms with high SI value PSR (red) to the top (based on the Splicing Index and p-value values of your highlighted PSR). Note the sorting order of the PSR and Isoforms. (Figure 2.44)

In the example below, (Figure 2.44)the top 2 isoforms plus the TR10004184.hg (some or all of them) are the likely isoforms in Liver (Condition1), because these isoforms contain 2 PSRs with a high Splicing Index. It also shows significant p-values in the Liver (Condition1) compare to Muscle (Condition2).

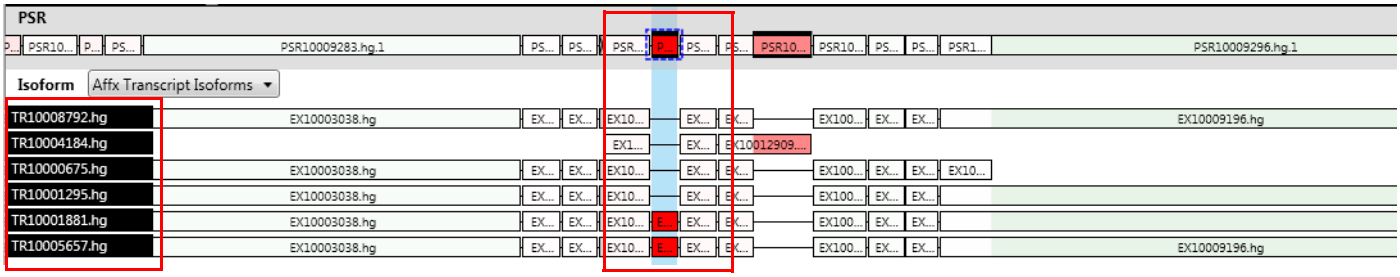
The bottom 2 isoforms plus TR10008792.hg (some or all of them) are the likely isoforms in Muscle (Condition2), because these isoforms do not contain these 2 PSRs.

Figure 2.44 .Up-Regulated on Top Sort Order Example



2. Click to highlight a PSR, then click Down-Regulated on Top button button to bring the isoforms with negative SI value PSR (green) to the top (based on the Splicing Index and p-value value of your highlighted PSR). Note the sorting order of the PSR and Isoforms. (Figure 2.45)

Figure 2.45 Down-Regulated on Top Sort Order Example



IMPORTANT: If Other than "Affx Transcript Isoforms" IDs are used, the software will do 2-level sorting. It first sorts Isoforms with IDs (for the selected source), then it performs a sub-sort within the sorted result.

Using the Show Junction Option

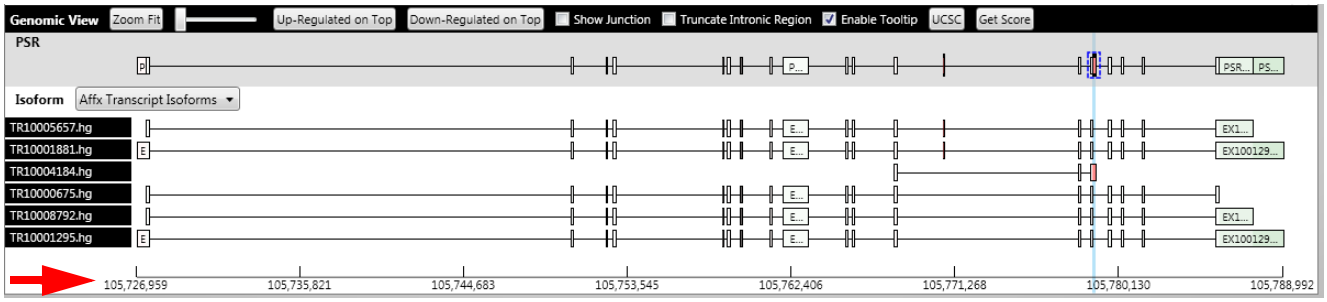
The Show Junction option is unchecked by default. Check it to produce dotted lines to show the relationship of the detected Junctions and PSRs. Uncheck this box to hide these junction lines.

Using the Truncate Intronic Region Option

The Truncate Intronic Region option is checked by default. It truncates the intronic regions to allow the exons to be more visible. Uncheck this box to show the true genomic locations of PSRs (exons) within a gene, including relative size and genomic coordinates. (Figure 2.46)

NOTE: The Genomic ruler (bottom) (Figure 2.46) only appears when the Truncate box is unchecked.

Figure 2.46 Un-truncated Intronic View



Using the Enable Tooltip Option

The Enable Tooltip option is checked by default. Mouse over (position the cursor over) a PSR or Junction to see the details related to that PSR or junction.

Using the Zoom Feature

1. **Zoom Method 1:** To zoom in on a region of interest, left-click, hold, then move the mouse to frame your selection. Release the mouse button.
2. **Zoom Method 2:** Left-click and hold onto the slider bar, then move it right to magnify your view. Use the horizontal scrolling bar to center your view.
3. **Undo:** Click Zoom Fit to return to the default view.

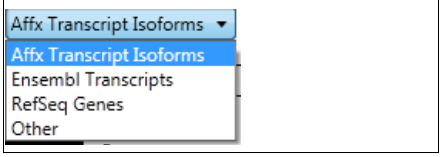
Selecting Different Transcript Isoforms IDs (Internet Connection Required)

By default, the transcript isoforms are displayed using Affymetrix Transcript Isoform IDs.

1. Click on the drop-down arrow, then click to select a different type of transcript isoform IDs. (Figure 2.47)

- Ensembl Transcripts: External public Transcript ID source.
- RefSeq Genes: External public RefSeq Transcript ID source.
- Other: Other public Transcript IDs.

Figure 2.47 Isoform drop-down menu



Linking Isoforms to an External Source (Internet Access Required)

1. Right-click on a Isoform of interest.

The following menu appears. (Figure 2.48)

Figure 2.48 Copy ID and Link to...Menu

2. Click on **Link to...** selection.

- Selecting RefSeq links to the NCBI website.
- Selecting Ensembl ID links to the Ensembl website.
- If the Isoform does not have a RefSeq or Ensembl ID associated with it, then linking to an external website is not available. If this is the case, click **Copy ID** to copy the Isoform ID to Windows Clipboard for pasting to another source.

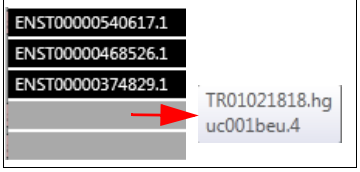
! NOTE: Available Link to... selections are depend on the Isoform selected from the Isoform drop-down menu. (Figure 2.47). Example: If Ensembl Transcripts is selected, then the *Link to NCBI* is grayed out (unavailable), as shown in Figure 2.48.

Your internet browser opens to the appropriate **(*ensembl.org or ncbi.gov*)** website with detailed information about your selection.

How Transcript Isoforms are Sorted

- Isoforms with IDs appear on top.
- Isoforms that fit a Condition1 criteria are sorted on top.
- Isoforms that fit a Condition2 criteria are sorted at the bottom.
- Choosing a different type of transcript isoform ID may leave some isoforms with blank gray boxes, because not every transcript isoform has a Ensembl ID or RefSeq ID. (Figure 2.49)
- Mouse over any Transcript ID to see all the associated IDs.

Figure 2.49 Gray Isoform



Linking Out to the UCSC Genome Browser (Internet Access Required)

Gene

1. Click to highlight a gene of interest, then click the UCSC button.

Your internet browser opens to the ***genomic.ucsc.edu*** website and displays detailed information about your selection.

Exons

1. Right-click on an Exon.

A menu appears.

2. Click **View Exon in UCSC**.

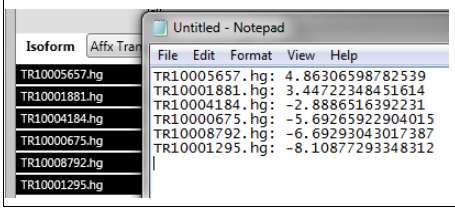
NOTE: You must click the Exon, not the PSR in order to link out.

Your internet browser opens to the ***genomic.ucsc.edu*** website and displays detailed information about your selected Exon.

Using the Get Score Button

1. Click the Get Score button to obtain the sorting scores. The scores appear in a Windows Notepad file. (Figure 2.50) See "How Transcript Isoforms are Sorted" on page 45.

Figure 2.50 Get Score Notepad File Example

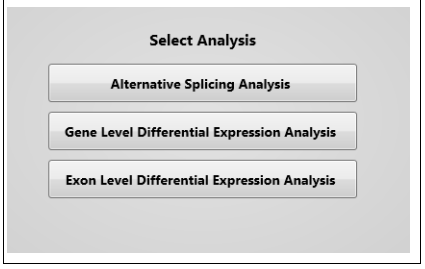


Gene Level Differential Expression Analysis

Setting Up an Analysis Using Gene CHP Files

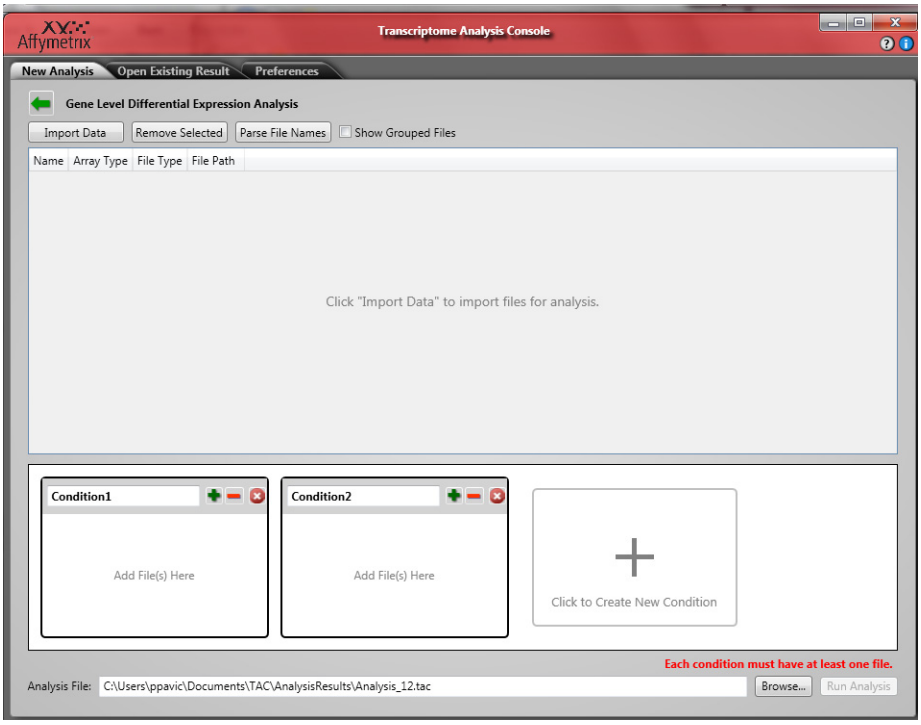
1. At the main TAC window, click **Gene Level Differential Expression Analysis.** (Figure 3.1).

Figure 3.1 Main TAC Window



2. The New Analysis window appears. (Figure 3.2)

Figure 3.2 New Analysis Window

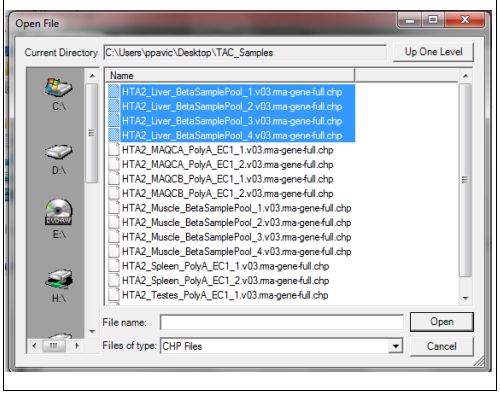


3. Click **Import Data**.

The following window appears. (Figure 3.3) It displays the data path you set up earlier and its files.

! NOTE: **The first time you launch TAC, it asks you to define a path to store your library and annotation files. For your convenience, TAC retains this path information. Affymetrix recommends you use the Expression Console library path you already configured.**

Figure 3.3 Import Data Window



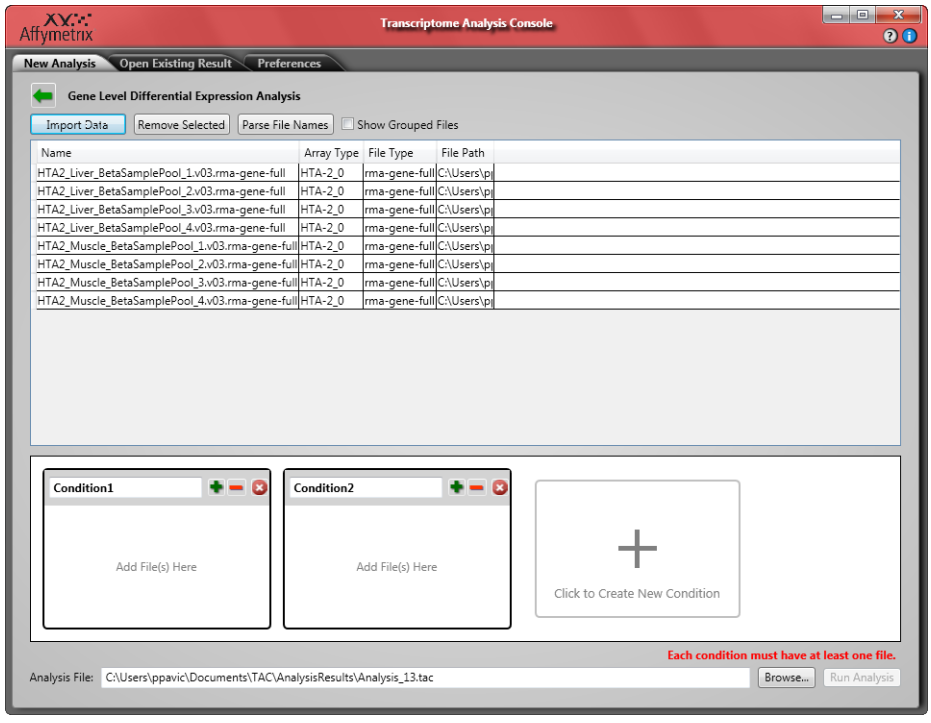
4. Single click, Ctrl click, or Shift click to select multiple files (as shown above).

! NOTE: To optimize the analysis, Affymetrix recommends importing more than 1 sample per condition.

5. Click **Open**.

The selected files are now populated in the Sample File Window. (Figure 3.4)

Figure 3.4 Import Data into Sample File Window



- Click Show Grouped Files checkbox (Figure 3.4) to display sample file names and attributes even they have been added to various conditions. Samples that have been added to different conditions get grayed out.
- Click Remove Selected (Figure 3.4) to remove a file(s) from the Sample File window.

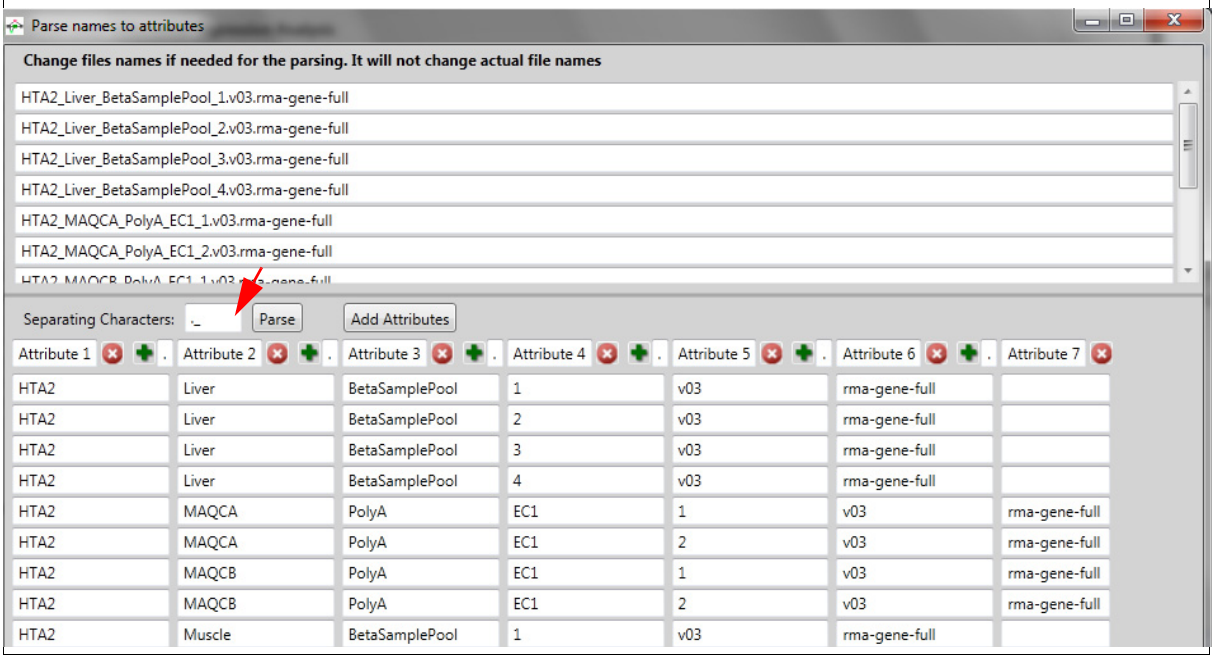
Parsing Imported Data File Names (Optional)

This option gives you the ability to parse attributes from the sample file names and helps you set up conditions.

1. Click Parse File Names.

The following window appears: (Figure 3.5)

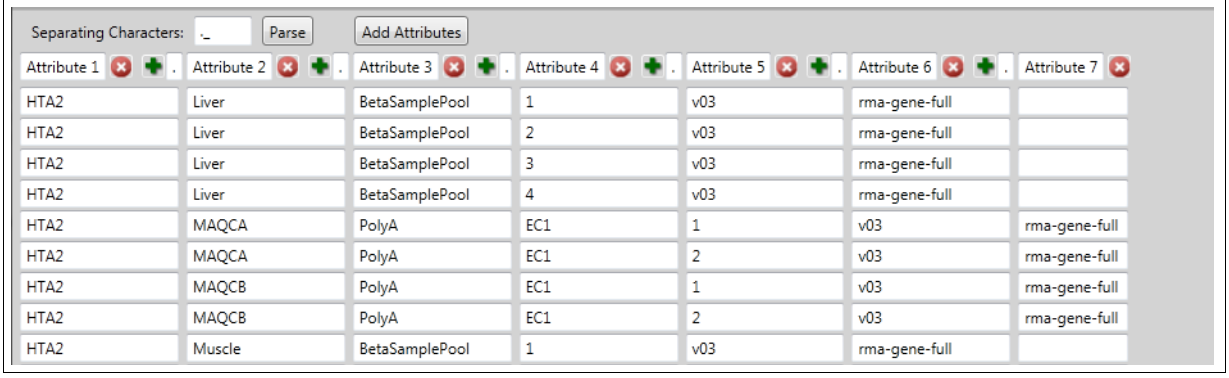
Figure 3.5 Parse Filename Window



2. Determine what common separating characters reside within your file names. The file name examples (in Figure 3.5) are separated by an underscore and period.
3. Type the appropriate symbols in the **Separating Characters** field. In this example, an underscore and period.
4. Click **Parse**.

The Parse File Names table now appears as shown. (Figure 3.6)

Figure 3.6 Parse Filenmae Table



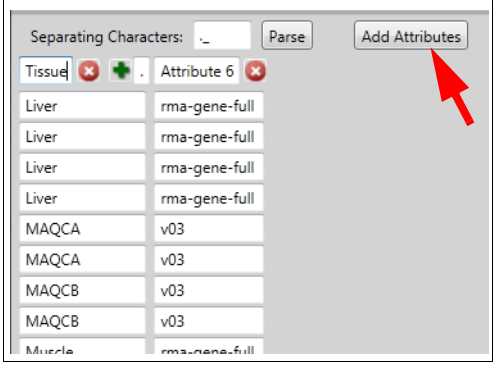
Do the following to clean up attributes parsed from the sample file names:

- Click inside any of the Attribute text fields Attribute 1 to type in a new Attribute name.

- Click . to enter a unique separating character for your parsed filename. The default separating character is a period. These characters are useful if you ever want to return a file to its original name.
- Click + to join together a neighboring attribute column.
- Click ⊗ to remove an attribute column from the table.

In the example above, since Attribute 1, 3, 4, 5, and 7 are redundant and not useful, they are removed. The table now appears as shown. (Figure 3.7)

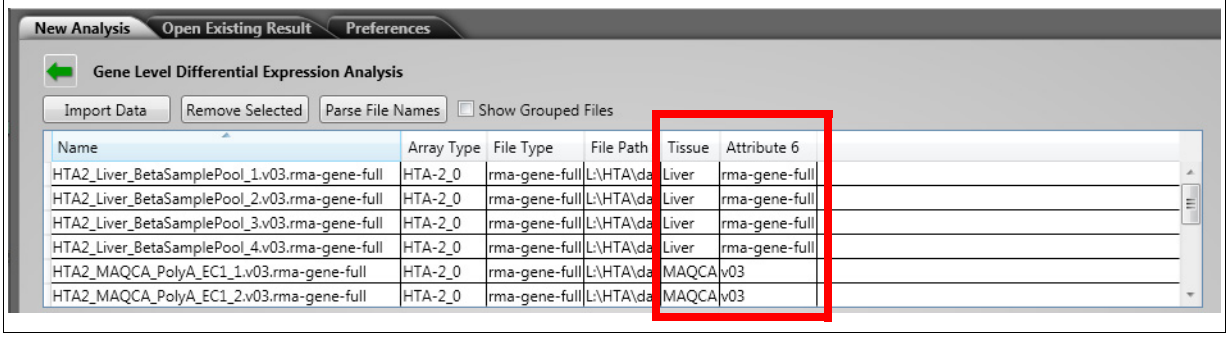
Figure 3.7 Parse Table with 2 Filenames



5. To save your parsed attributes to the Same File window, click **Add Attributes**.

The parsed file name attributes (Figure 3.7) are added to the Sample File window as additional attributes. (Figure 3.8)

Figure 3.8 Parsed Results shown in the Sample File Window



Importing CHP Files into Different Condition Groups

! IMPORTANT: Customize your condition names first, then add the CHP files into each condition.

Do the following to populate the Condition1 window:

1. Click on the **Condition1** window header field to rename it to an appropriate Condition name.
2. Click to select and highlight the data you want to use for Condition1.
3. Click + in the Condition1 window to add your selected files to the Condition1 window.
4. If needed, click − to move selected files back to the Sample File window.
5. If needed, click ⊗ to delete your current Condition and move all its files back to the Sample File window.

Importing Files using Drag and Drop

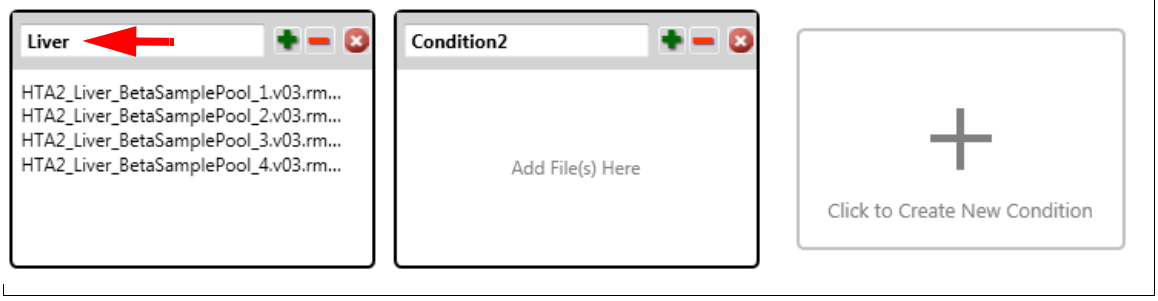
Do the following to drag and drop files into a Condition window:

1. Click Shift or click Ctrl, then click to select a group of files.
2. Click and hold onto the last file in the group, then drag them into the appropriately labeled Condition window.
3. Release the mouse button.

The Condition1 (Liver) window now contains your files.

4. If needed, click to move the file(s) back to the Sample File window.

Figure 3.9 Newly Labeled Condition1 with Samples



5. Repeat the steps 1-4 (above) for Condition2.
6. To create more than 2 conditions (recommended), click **Click to Create New Condition** (Figure 3.9), then repeat steps 1-4 (above) for your 3rd Condition.
7. If needed, edit your Analysis result file path and/or name by clicking inside the **Analysis File** text field (Figure 3.10), or click **Browse** to select a new file destination.

Figure 3.10 Analysis File Field

8. After the Conditions have been labeled and populated, click Run Analysis.

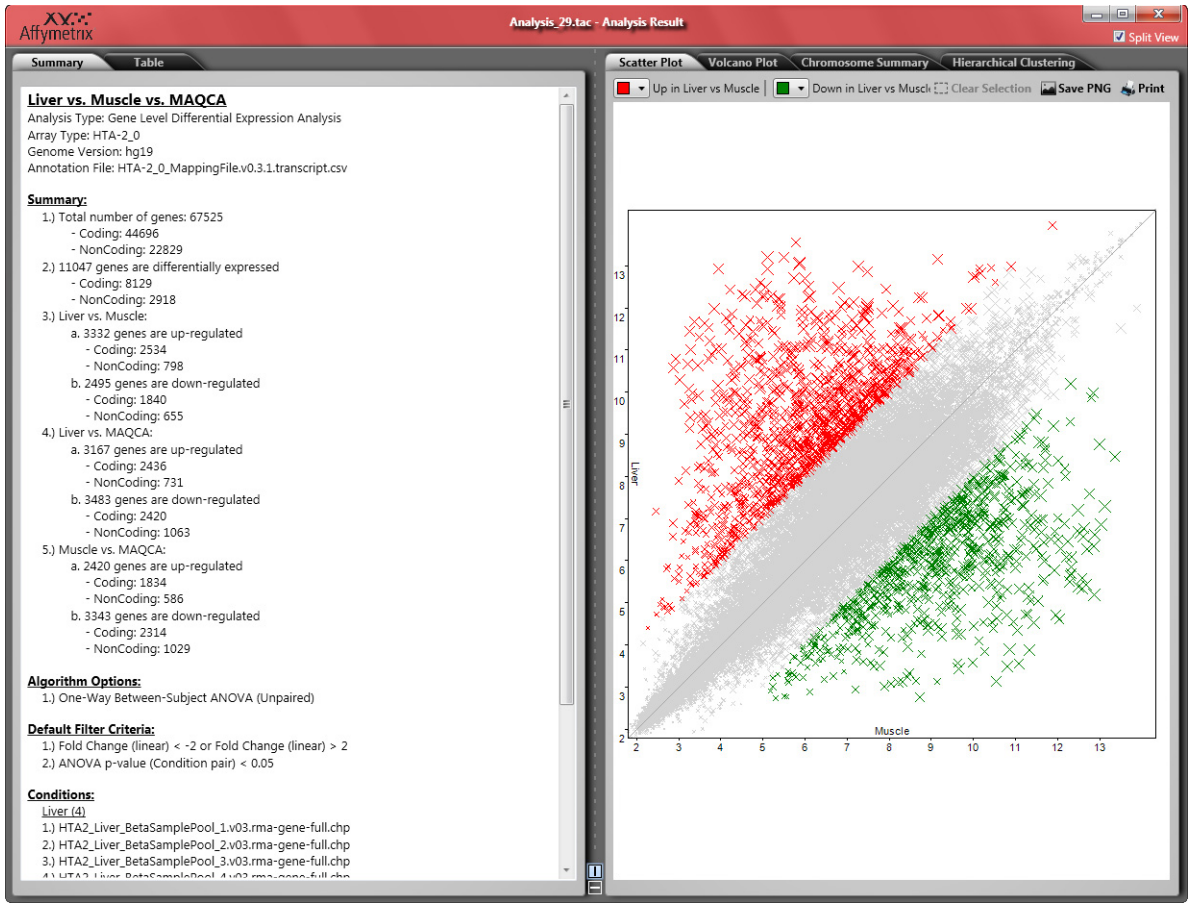
! TIP: TAC auto-saves your studies. At any time, click on the Open Existing Result tab to view recent analysis results.

Please Wait... appears. then the Analysis Result viewer appears. By default, this new viewer opens in a split-screen configuration. (Figure 3.11)

By default, the Summary tab and Scatter Plot graph tabs appear side by side.

! NOTE: Click to uncheck the Split View checkbox to display each window as a full screen.

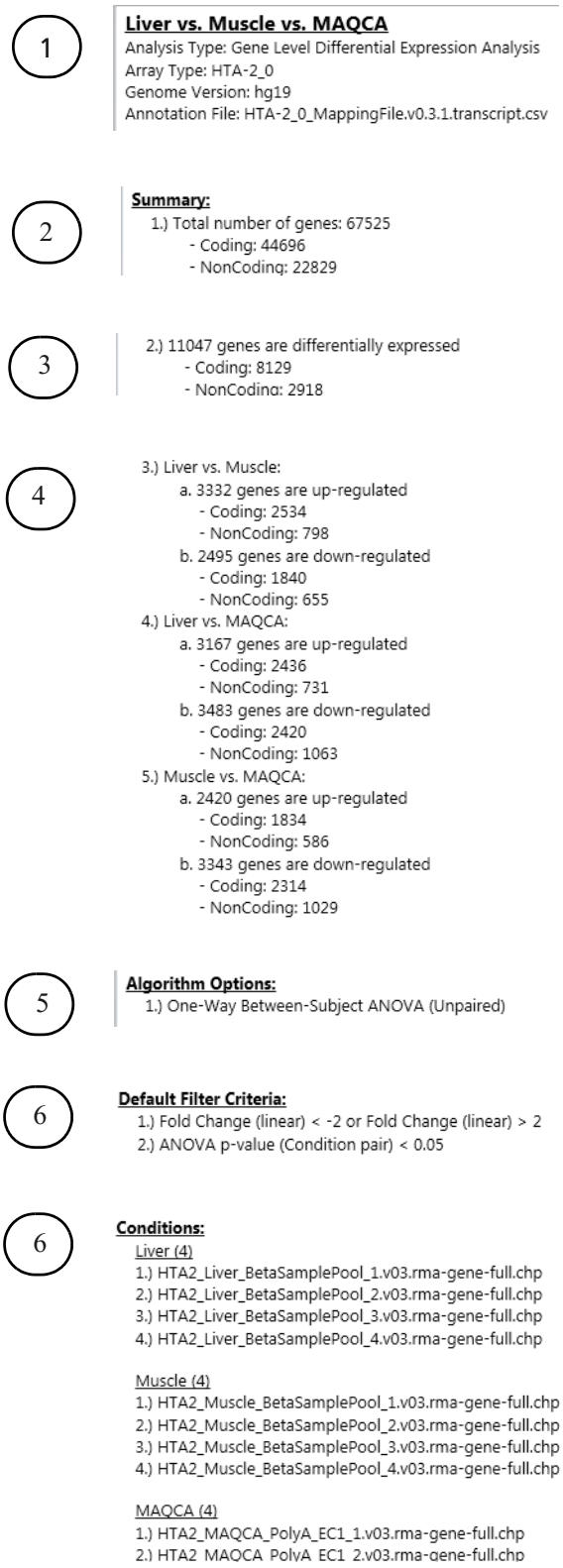
Figure 3.11 Default View (after running an analysis)



Gene Level Differential Expression Analysis Summary Window (Overview)

The Summary information is static and based on the algorithm parameters applied during the analysis. Below is an example of the information collected in the Summary window. (Figure 3.12)

Figure 3.12 Summary window



1. Summary information per this analysis. NOTE: Summaries vary between Gene, Exon, and Splicing analysis.
2. Array summary. Total number of transcript clusters, and numbers of coding and noncoding transcript clusters on this array.
3. Summary of total differentially expressed transcript clusters among all conditions (union of differentially expressed transcript clusters from all unique condition pairs) Also displays the summary of total differentially expressed Coding and Non-Coding Transcript Clusters.
4. Summary of total differentially expressed transcript clusters per condition pair.These are the Coding and NonCoding transcript clusters that pass the default filtering criteria listed in the "Default Filter Criteria" section below.

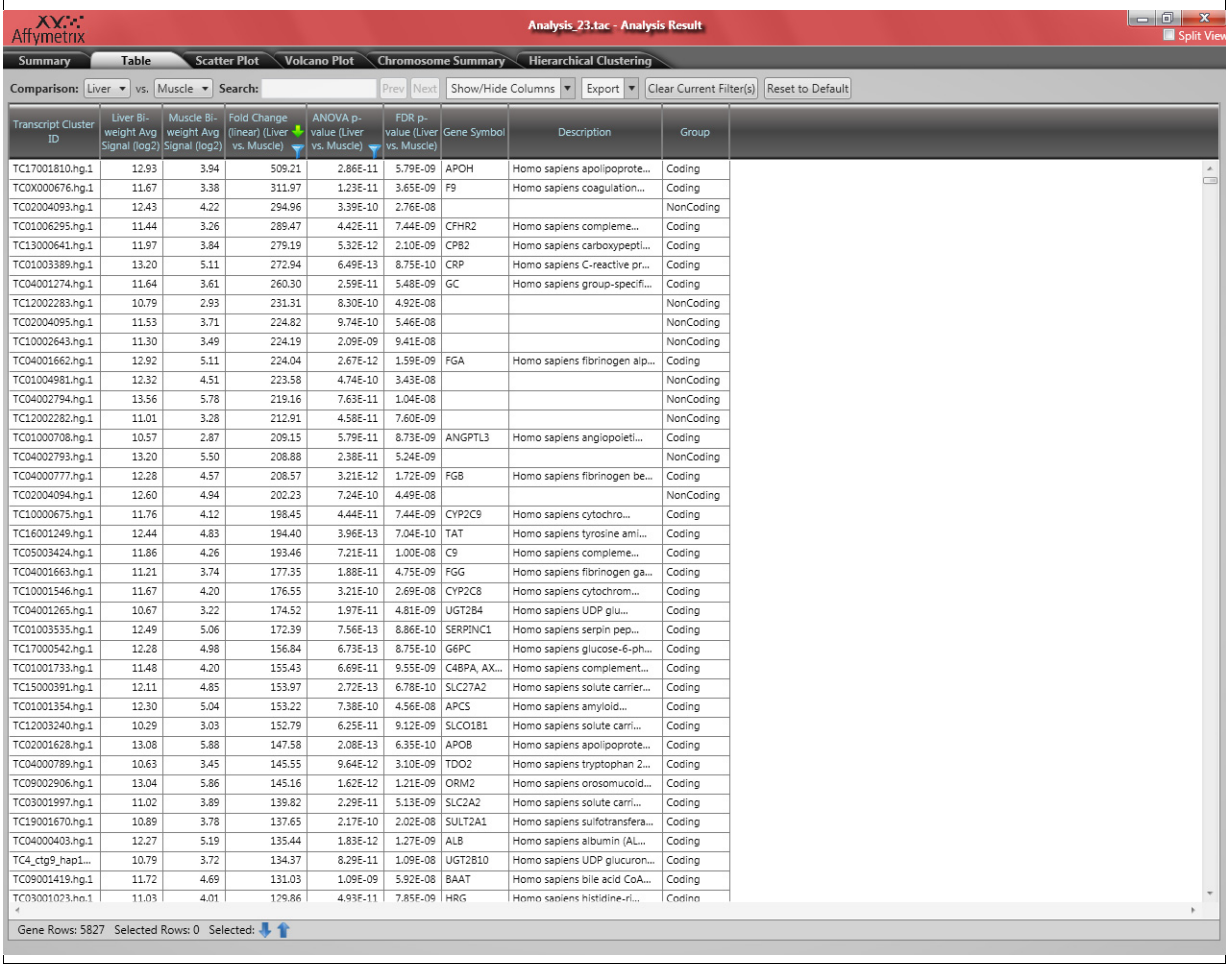
   Shows total number of up-regulated Coding and Non-Coding transcript clusters in this condition pair.

   Shows total number of down-regulated Coding and Non-Coding transcript clusters in this condition pair.
5. Shows the algorithm used to perform the Gene Level Differential Expression Analysis.
6. This section displays the factory default filtering criteria results. NOTE: Only transcript clusters that pass through these criteria are summarized in the "Summary" section above and listed in the Gene table as default.
7. Displays each Condition name and the total number of CHP files in it. Note: Scroll the Summary window downward to reveal the other Conditions in your analysis.

Gene Level Differential Expression Analysis Table Window (Overview)

1. After reviewing the Summary, click the **Table** tab or click to uncheck the Split View checkbox to display the Table in full screen. (Figure 3.13)

Figure 3.13 Gene Table - Full Screen



Transcript Cluster ID	Liver Bi-weight Avg Signal (log2)	Muscle Bi-weight Avg Signal (log2)	Fold Change (linear) (Liver vs. Muscle)	ANOVA p-value (Liver vs. Muscle)	FDR p-value (Liver vs. Muscle)	Gene Symbol	Description	Group
TC17001810.hg.1	12.93	3.94	509.21	2.86E-11	5.79E-09	APOH	Homo sapiens apolipoprote...	Coding
TC0X000676.hg.1	11.67	3.38	311.97	1.23E-11	3.65E-09	F9	Homo sapiens coagulation...	Coding
TC02004093.hg.1	12.43	4.22	294.96	3.39E-10	2.76E-08			NonCoding
TC01006295.hg.1	11.44	3.26	289.47	4.42E-11	7.44E-09	CFHR2	Homo sapiens compleme...	Coding
TC13000641.hg.1	11.97	3.84	279.19	5.32E-12	2.10E-09	CPB2	Homo sapiens carboxypepti...	Coding
TC01003389.hg.1	13.20	5.11	272.94	6.49E-13	8.75E-10	CRP	Homo sapiens C-reactive pr...	Coding
TC04001274.hg.1	11.64	3.61	260.30	2.59E-11	5.48E-09	GC	Homo sapiens group-specifi...	Coding
TC12002283.hg.1	10.79	2.93	231.31	8.30E-10	4.92E-08			NonCoding
TC02004095.hg.1	11.53	3.71	224.82	9.74E-10	5.46E-08			NonCoding
TC10002643.hg.1	11.30	3.49	224.19	2.09E-09	9.41E-08			NonCoding
TC04001662.hg.1	12.92	5.11	224.04	2.67E-12	1.59E-09	FGA	Homo sapiens fibrinogen alp...	Coding
TC01004981.hg.1	12.32	4.51	223.58	4.74E-10	3.43E-08			NonCoding
TC04002794.hg.1	13.56	5.78	219.16	7.63E-11	1.04E-08			NonCoding
TC12002282.hg.1	11.01	3.28	212.91	4.58E-11	7.60E-09			NonCoding
TC01000708.hg.1	10.57	2.87	209.15	5.79E-11	8.73E-09	ANGPTL3	Homo sapiens angiopoieti...	Coding
TC04002793.hg.1	13.20	5.50	208.88	2.38E-11	5.24E-09			NonCoding
TC04000777.hg.1	12.28	4.57	208.57	3.21E-12	1.72E-09	FGB	Homo sapiens fibrinogen be...	Coding
TC02004094.hg.1	12.60	4.94	202.23	7.24E-10	4.49E-08			NonCoding
TC10000675.hg.1	11.76	4.12	198.45	4.44E-11	7.44E-09	CYP2C9	Homo sapiens cytochro...	Coding
TC16001249.hg.1	12.44	4.83	194.40	3.96E-13	7.04E-10	TAT	Homo sapiens tyrosine ami...	Coding
TC05003424.hg.1	11.86	4.26	193.46	7.21E-11	1.00E-08	C9	Homo sapiens compleme...	Coding
TC04001663.hg.1	11.21	3.74	177.35	1.88E-11	4.75E-09	FGG	Homo sapiens fibrinogen ga...	Coding
TC10001546.hg.1	11.67	4.20	176.55	3.21E-10	2.69E-08	CYP2C8	Homo sapiens cytochrom...	Coding
TC04001265.hg.1	10.67	3.22	174.52	1.97E-11	4.81E-09	UGT2B4	Homo sapiens UDP glu...	Coding
TC01003535.hg.1	12.49	5.06	172.39	7.56E-13	8.86E-10	SERPINC1	Homo sapiens serpin pep...	Coding
TC17000542.hg.1	12.28	4.98	156.84	6.73E-13	8.75E-10	G6PC	Homo sapiens glucose-6-ph...	Coding
TC01001733.hg.1	11.48	4.20	155.43	6.69E-11	9.55E-09	C4BPA, AX...	Homo sapiens complement...	Coding
TC15000391.hg.1	12.11	4.85	153.97	2.72E-13	6.78E-10	SLC27A2	Homo sapiens solute carrier...	Coding
TC01001354.hg.1	12.30	5.04	153.22	7.38E-10	4.56E-08	APCS	Homo sapiens amyloid...	Coding
TC12003240.hg.1	10.29	3.03	152.79	6.25E-11	9.12E-09	SLCO1B1	Homo sapiens solute carri...	Coding
TC02001628.hg.1	13.08	5.88	147.58	2.08E-13	6.35E-10	APOB	Homo sapiens apolipoprote...	Coding
TC04000789.hg.1	10.63	3.45	145.55	9.64E-12	3.10E-09	TDO2	Homo sapiens tryptophan 2...	Coding
TC09002906.hg.1	13.04	5.86	145.16	1.62E-12	1.21E-09	ORM2	Homo sapiens orosomucoid...	Coding
TC03001997.hg.1	11.02	3.89	139.82	2.29E-11	5.13E-09	SLC2A2	Homo sapiens solute carri...	Coding
TC19001670.hg.1	10.89	3.78	137.65	2.17E-10	2.02E-08	SULT2A1	Homo sapiens sulfotransfera...	Coding
TC04000403.hg.1	12.27	5.19	135.44	1.83E-12	1.27E-09	ALB	Homo sapiens albumin (AL...	Coding
TC4_ctg9_hap1...	10.79	3.72	134.37	8.29E-11	1.09E-08	UGT2B10	Homo sapiens UDP glucuron...	Coding
TC09001419.hg.1	11.72	4.69	131.03	1.09E-09	5.92E-08	BAAT	Homo sapiens bile acid CoA...	Coding
TC03001023.hg.1	11.03	4.01	129.86	4.93E-11	7.85E-09	HRG	Homo sapiens histidine-ri...	Coding

Parts of the Table

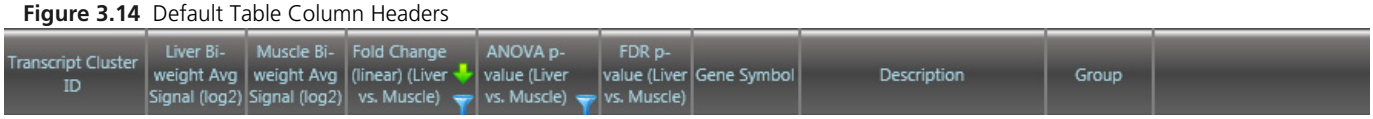
- Column Headers
- Table Options

Column Headers

The factory default columns and 2 preset filters are as shown: (Figure 3.14)

See Table 3.1 for definitions of these columns.

Figure 3.14 Default Table Column Headers



Showing or Hiding Table Columns

1. Click the Show/Hide Columns drop-down menu to show or hide columns in the splicing table.
2. Click outside the Show/Hide Drop-down menu to close it.

Table 3.1 Gene Table Columns and their Definitions (Column entries in BOLD are factory defaults)

Available Columns	Description
Transcript Cluster ID	ID of Transcript Cluster (TC)
(Condition1) Bi-weight Avg Signal (log2) **(Condition2) Bi-weight Avg Signal (log2)**	Tukey's Bi-weight[1] average of gene intensity of all the samples in this condition: Bi-weight average of (sample 1 gene1 intensity + sample 2 gene1 intensity +…+ sample N gene1 intensity.
(Condition1) Standard Deviation (Condition2) Standard Deviation	Standard Deviation[2] of gene intensities from all samples in this condition: STDEV of (sample 1 gene1 intensity + sample 2 gene1 intensity +…+ sample N gene1 intensity)
Gene Fold Change (linear) (Condition1 vs. Condition2)	This shows the fold change (in linear space) of Condition1 vs. Condition2. $2^{[\text{Condition1 Gene Avg Signal (log2) - Condition2 Gene Avg Signal (log2)}]}$
ANOVA p-value (Condition1 vs. Condition2)	One-Way Between-Subject ANOVA[2] p-value (Condition 1 vs. Condition2)
FDR p-value (Condition1 vs. Condition2)	FDR adjusted p-value based on Benjamini-Hochberg Step-Up FDR-controlling Procedure[5] IMPORTANT: All ANOVA p-values from all genes are sent to FDR for correction.
ANOVA p-value (All Conditions)	One-Way Between-Subject ANOVA p-value for all conditions.
FDR p-value (All Conditions)	FDR adjusted p-value based on Benjamini-Hochberg Step-Up FDR-controlling Procedure[5] for all conditions. IMPORTANT: All ANOVA p-values from all genes are sent to FDR for correction.
Gene Symbol	Gene symbol for this transcript cluster. Note: RefSeq gene symbol is listed as the first gene symbol (if there are more than 1 gene symbol).
Description	Gene Description for this TC.
Chromosome	Chromosome for this transcript cluster. See Chromosome Naming Scheme[3] for a detailed description.
Genomic Position	Genomic Start/Stop position for this TC.
Public Gene IDs	Public Gene IDs for this TC.
Group	Whether this TC is coding, non-coding, or other.

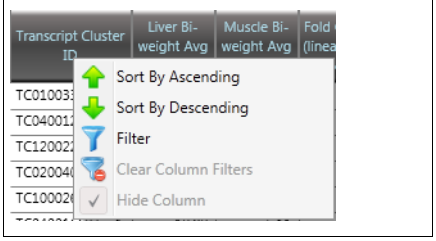
Sorting Columns

Right-Click Method

1. Select a column, then right-click on it.

The following window appears: (Figure 3.15)

Figure 3.15 Right-click Column Menu



2. Click to select either **Sort By Ascending** (A-Z) or **Sort By Descending** (Z-A).

Double-Click Method

1. Double-click on a column header to sort its data in an ascending order. Double-click on the same column header to sort its data in a descending order.

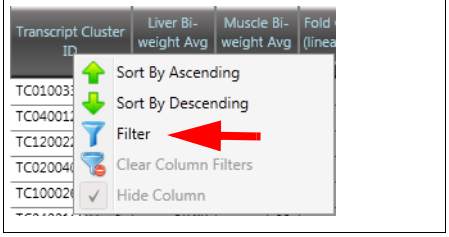
Filtering Column Data

All table columns are filterable.

1. Select a column, then right-click on it.

The following window appears: (Figure 3.15)

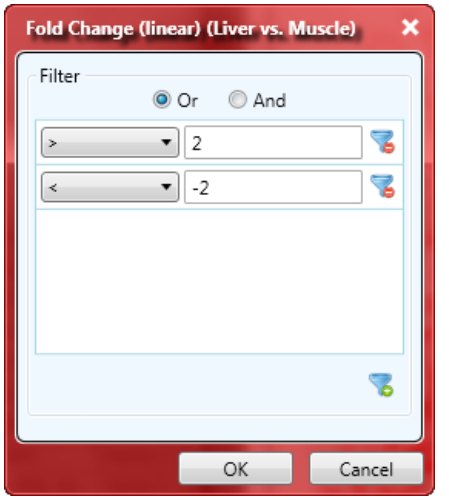
Figure 3.16 Right-click Column Menu



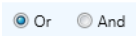
2. Click **Filter**.

The following window appears (Fold Change column example shown): (Figure 3.17)

Figure 3.17 Filter Properties



Editing Filtering Properties:

1. Click the **Or** or **And** button to choose **Or** or **AND** logic. 

2. Click the symbol drop-down menu(s) to select new symbol(s).(Figure 3.18)

Figure 3.18 Drop-down Menu

3. Click inside the numbering field(s)  to enter new value(s).

4. Click  to add filter(s).
5. Click  to remove filter(s).

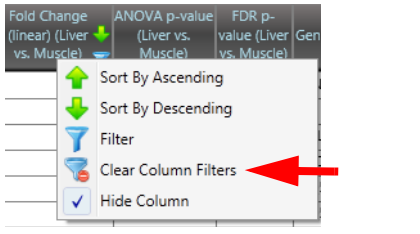
Clearing Filters

Individual Filter

1. Right-click on the filtered column you want to clear.

The following window appears: (Figure 3.19)

Figure 3.19 Right-click Column Menu



2. Click **Clear Column Filters**.

The filter is removed.

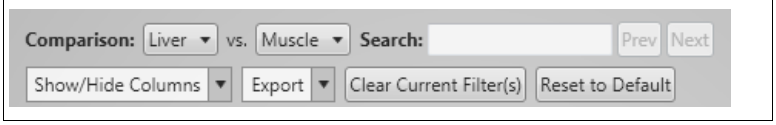
All Filters

1. Click Clear Current Filter(s) remove **ALL** filters from the table headers.

Table Options

Use the Table Options Menu (Figure 3.19) to customize your table view.

Figure 3.20 Table options menu



Rearranging Factory Set Columns

1. Click on a column you want to move.
2. Drag it (left or right) to its new location.
3. Release the mouse button.
4. The column is now in its new position.

Reset to Default

1. Click Reset to Default to return the table to its factory setting.

Searching Keywords

NOTE: The Search Tool is limited to finding matching strings. It is not a full search engine.

1. To search for a keyword within your table, click inside the **Search** field, then type your keyword.
2. Click the Prev or Next buttons to search. (Figure 3.21)

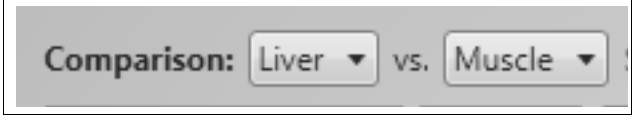
Figure 3.21 Search Tool

Changing Condition vs. Condition Pairings

1. Use the **Comparison** drop-down menus to change your condition pairings. (Figure 3.22)

You **must** choose 2 different conditions. Identical condition pairings generates the error message, ***Please Choose Two Different Conditions***

Figure 3.22 Condition Pairing Drop-down



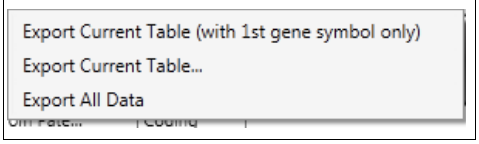
IMPORTANT: Table and graph results ONLY reflect your current Condition pairing.

Exporting Options

If you want to export (Save) your analysis table, click Export drop-down.

The following Export options appear: (Figure 3.23)

Figure 3.23 Export Menu



Exporting the Current Table with 1st Gene Symbols

NOTE: This option shows the first gene symbol only if there is more than one gene symbol for the selected transcript cluster.

1. Click **Export Current Table (with 1st gene symbol only)**.

The **Save As** window appears.

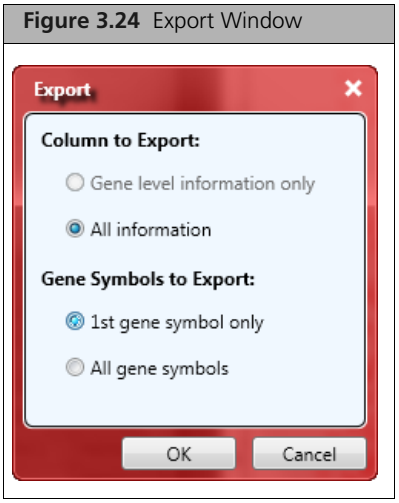
2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting the Current Table

1. Click **Export Current Table**.

The following window appears: (Figure 3.24)

Figure 3.24 Export Window



Column to Export

1. Click either **Gene level information only** or **All information**.

Gene Symbols to Export

1. Click either **1st gene symbol** or **All gene symbols**
2. Click **OK**

The **Save As** window appears.

3. Click on an existing folder or click **New Folder** to choose a new save location.
4. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting All Data

1. Click **Export All Data**.

! NOTE: Only currently paired data is exported, including data in the hidden columns, and the paired data's gene level information.

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

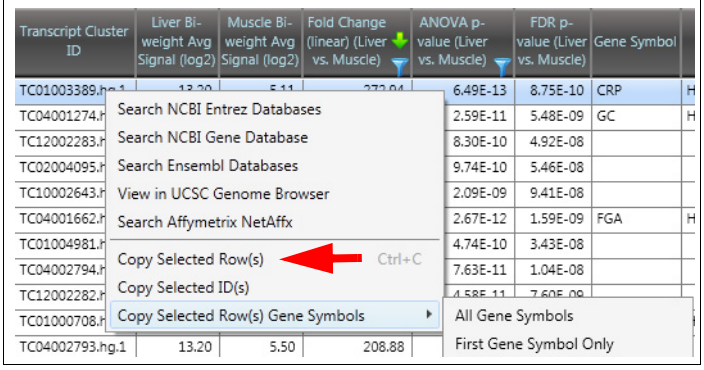
Saving Table Information

Use this copy feature to save table information to the Windows Clipboard, then use this buffered information for pasting into other applications or websites.

Copy Selected Row(s)

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.
2. Right-click, then click to select **Copy Selected Row(s)**. (Figure 3.25)

Figure 3.25 Copy Selected Row(s) option

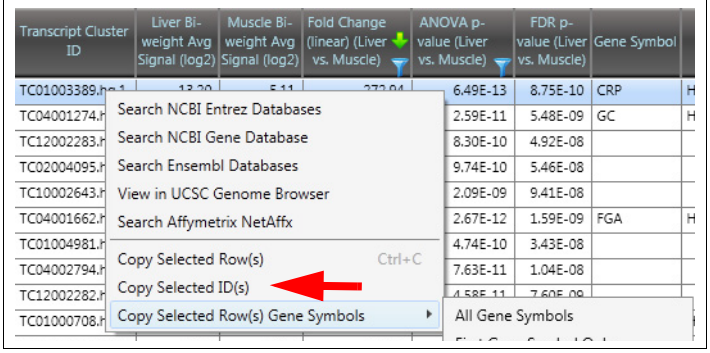


The selected gene level information (shown on the left side of the splicing table) are now copied to the Windows Clipboard for pasting.

Copy Selected ID(s)

1. Click to highlight (light blue) a ID or Ctrl left-click to highlight multiple rows.
2. Right-click, then click **Copy Selected ID(s)** to copy Transcript Cluster IDs). (Figure 3.26)

Figure 3.26 Copy Selected ID(s) option



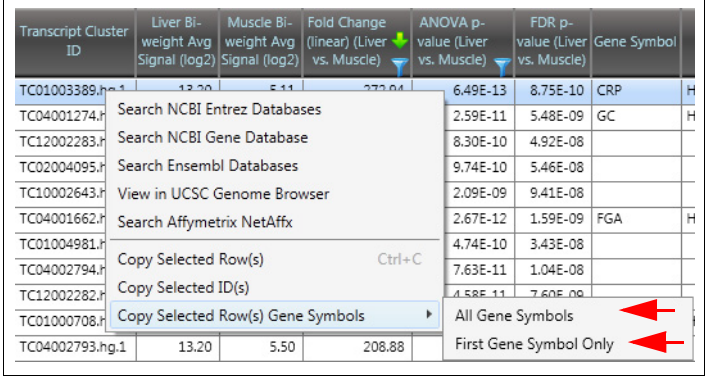
The selected TC IDs are now copied to the Windows Clipboard for pasting.

Copy Selected Row(s) Gene Symbols

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.
2. Right-click on the selection, then click to select **Copy Selected Row(s) Gene Symbols**.

3. Click to select either **All Gene Symbols** (all possible gene symbols for a Transcript Cluster) or **First Gene Symbol Only** (the first gene symbol that belongs to the Transcript Cluster). (Figure 3.27)

Figure 3.27 Copy Selected Row(s) gene Symbols options



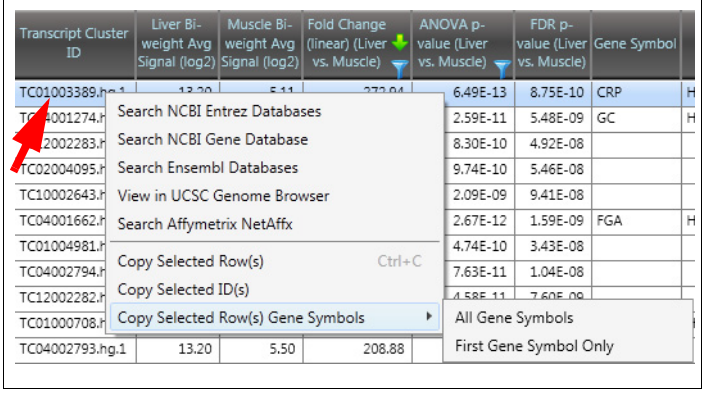
Your selected rows with gene symbols are now copied to the Windows Clipboard.

Accessing External Databases (Internet Connection Required)

1. To link out to various external databases, right-click on a TC of interest.

The following menu appears: (Figure 3.28

Figure 3.28 Search Database menu



2. Click to select the external database you want to visit.

Your internet browser opens to the appropriate website.

Searching the Affymetrix NetAffx Website

1. Click to select **Search Affymetrix NetAffx**.

The internet browser opens to the Affymetrix Customer Login window.

2. Enter your NetAffx **Email ID** and **Password**, then click **Submit**.

Your internet browser opens to the ***Netaffx Query Center*** and displays information about your gene of interest.

! NOTE: The NetAffx Query Center is compatible with Windows Internet Explorer and Firefox. Chrome is not supported at this time.

NOTE: If a Probe Set or Transcript Cluster is not available, an appropriate message appears.

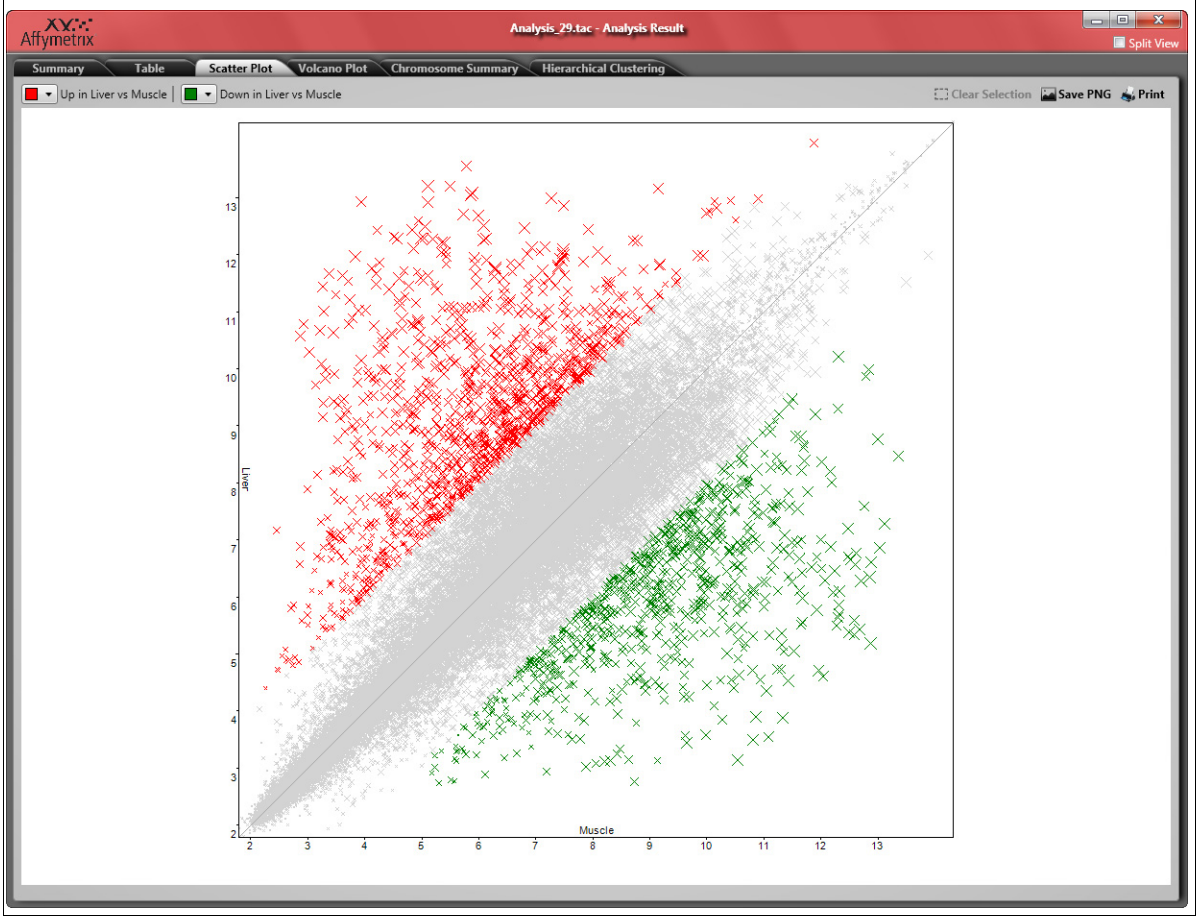
Gene Level Differential Expression Analysis Graphs

NOTE: Scatter Plot, Volcano Plot, Chromosome Summary Graphs, Gene Tables, and your selections stay in sync with each other. The data displayed is always based on your current Condition pairings.

Scatter Plot Graph (Overview)

- The Scatter Plot is a standard scatter plot graph of your current condition paring. (Figure 3.29)
- The size of the X is based on p-value, the smaller p-value, the bigger the X;
- The signal is log2 based data. The scale is also log2 based scale for signals.
- X axis is second condition. The Y axis is first condition on the top left of the table.
- The gray TCs are the ones filtered out by the table. The green TCs are the ones down-regulated in Liver (Condition1) vs. Muscle (Condition2). The red TCs are the ones up-regulated in Liver (Condition1) vs. Muscle (Condition2).
- If you switch the condition pair or the filter criteria in the table, the data in the graph will change accordingly.

Figure 3.29 Scatter Plot Graph



Lassoing Genes of Interest

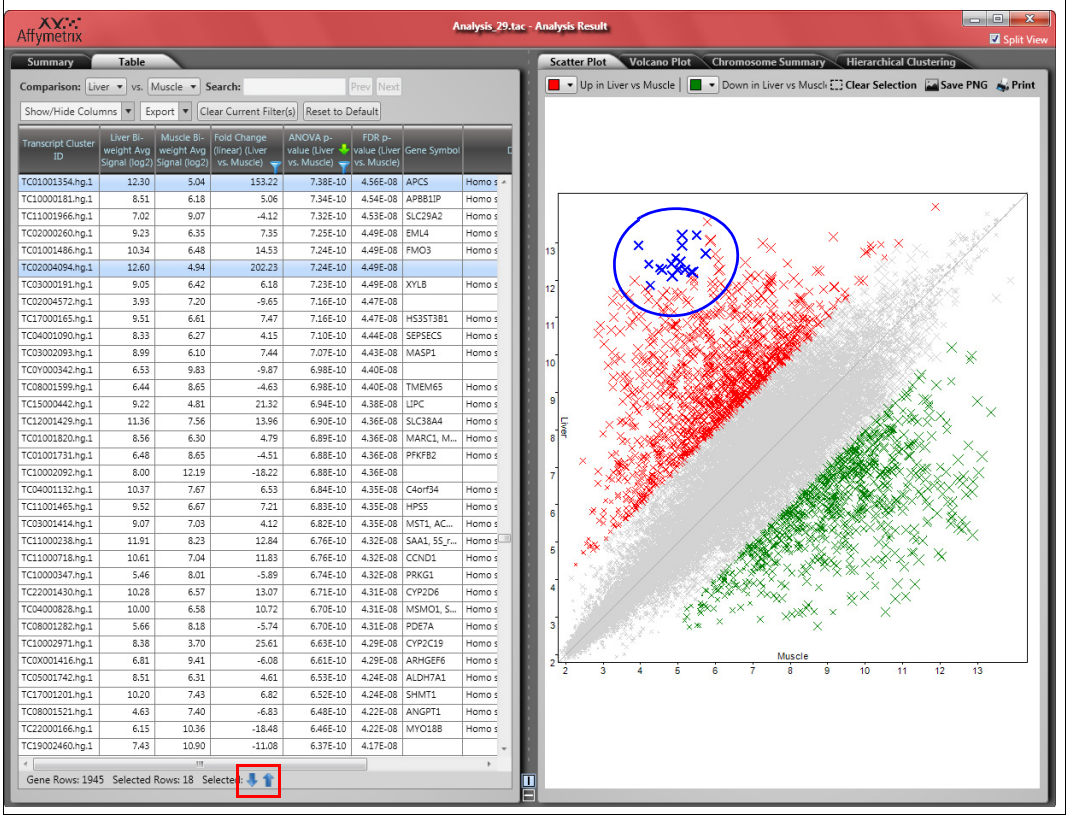
The Lasso tool allows you to select genes of interest inside the scatter plot.

1. To lasso a gene or a group of genes, hold down the left mouse button and use the ***cross hair*** cursor to encircle the gene(s) of interest. (Figure 3.30)

Once a complete circle is made, the genes of interest are highlighted in blue.

The data for these genes are also highlighted (blue) inside the table view, as shown below.

Figure 3.30 Scatter Plot Graph and Table



2. Click the **blue** up or down arrows (shown) to navigate through each highlighted gene or right click in the table to get the gene symbols/IDs of these lassoed genes.

Using the Table to Investigate Lassoed Selections

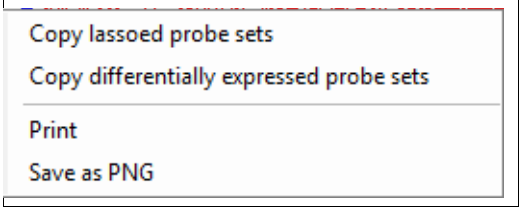
See "Saving Table Information" on page 61 to search and copy your lassoed selection.

Copying Lassoed Selections

1. Right-click on the graph.

The following menu appears. (Figure 3.31)

Figure 3.31 Right-click Graph Menu



Copy lassoed probe sets

1. Click **Copy lassoed probe sets** to copy your currently lassoed probes to the Windows Clipboard for pasting.

Copy differentially expressed probe sets

1. Click **Copy differentially expressed probe sets** to the Windows Clipboard for pasting.

Print

1. Click **Print** to print the graph to a pre-configured printer.

The Print window appears.

2. Configure the printing options as you normally would, then click **OK**.

Save as PNG

1. Click **Save PNG** to save the graph as a .PNG image file

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the graph, then click **Save**. The graph is now saved as a **.png** file.

Clearing Lassoed Selections

This option is enabled after genes have been lassoed as points of interest.

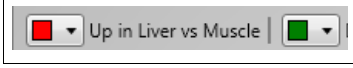
1. Click Clear Selection to clear lassoed genes from the graph and table.

! TIP: You can also clear a lassoed selection by lassoing a white (blank) space within the graph.

Changing Graph Colors

Use the drop-down menus to select your up and down regulated probe set graph colors. (Figure 3.31)

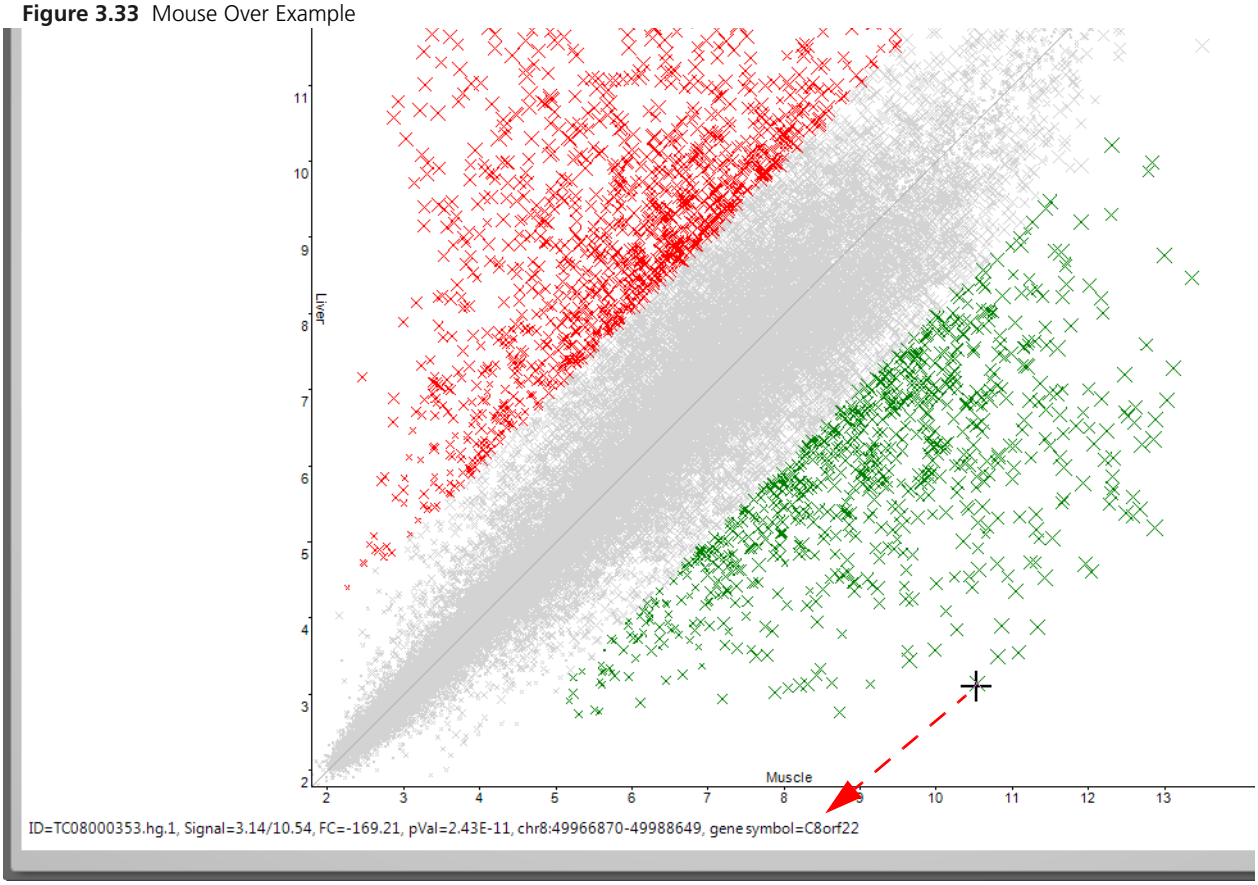
Figure 3.32 Graph Color Menus (Red and Green are default)



Obtaining Information Related to Individual Probe Sets

1. Mouse over (position the cursor over) a probe set to show its details. In the example below, (Figure 3.33) signals from both conditions (3.14 is the signal in Liver (Condition1) and 10.54 is the signal in Muscle (Condition2)) are shown, Fold change (FC) in linear scale, ANOVA p-value, chromosome positions and gene symbol are also identified in the line below (Figure 3.33).

Figure 3.33 Mouse Over Example



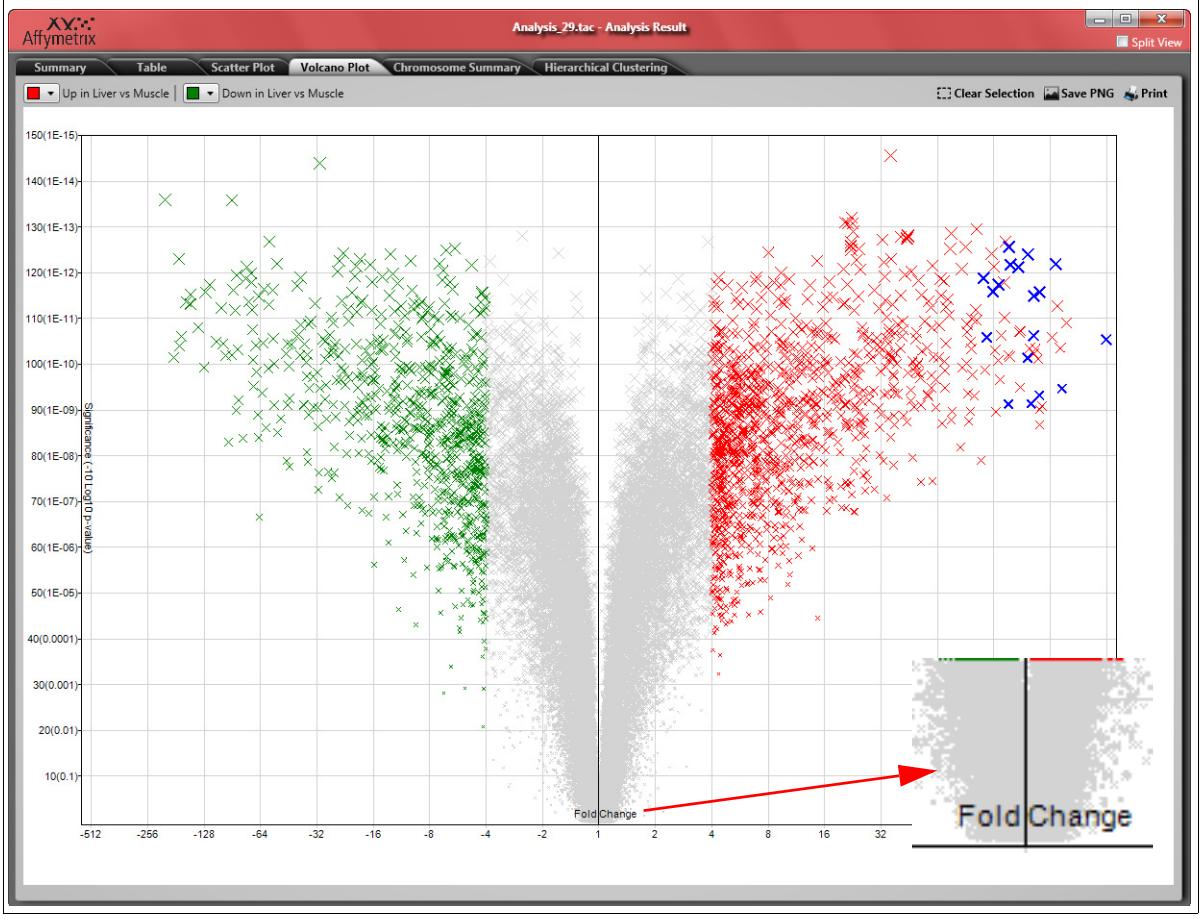
TIP: The best way to view individual probe sets is to make the graph full screen by un-checking Split View

Volcano Plot Graph (Overview)

- The Volcano Plot graph is a type of scatter plot that is used to quickly identify changes in large datasets. It plots significance versus fold-change on the y- and x-axes, respectively.
- X axis is the linear fold change from current condition pair; Y axis is -10log10 p-value of the ANOVA p-values.
- In the example below (Figure 3.34), The gray TCs are the ones filtered out by the table. The green TCs are down-regulated in Liver (Condition1) vs. Muscle (Condition2), while the red TCs are represented as up-regulated in Liver (Condition1 vs. Muscle (Condition2).

- If you switch the condition pair or the filter criteria in the table, the data in the graph will change accordingly.

Figure 3.34 Volcano Plot Graph



Lassoing Genes of Interest

The Lasso tool allows you to select genes of interest inside the volcano plot.

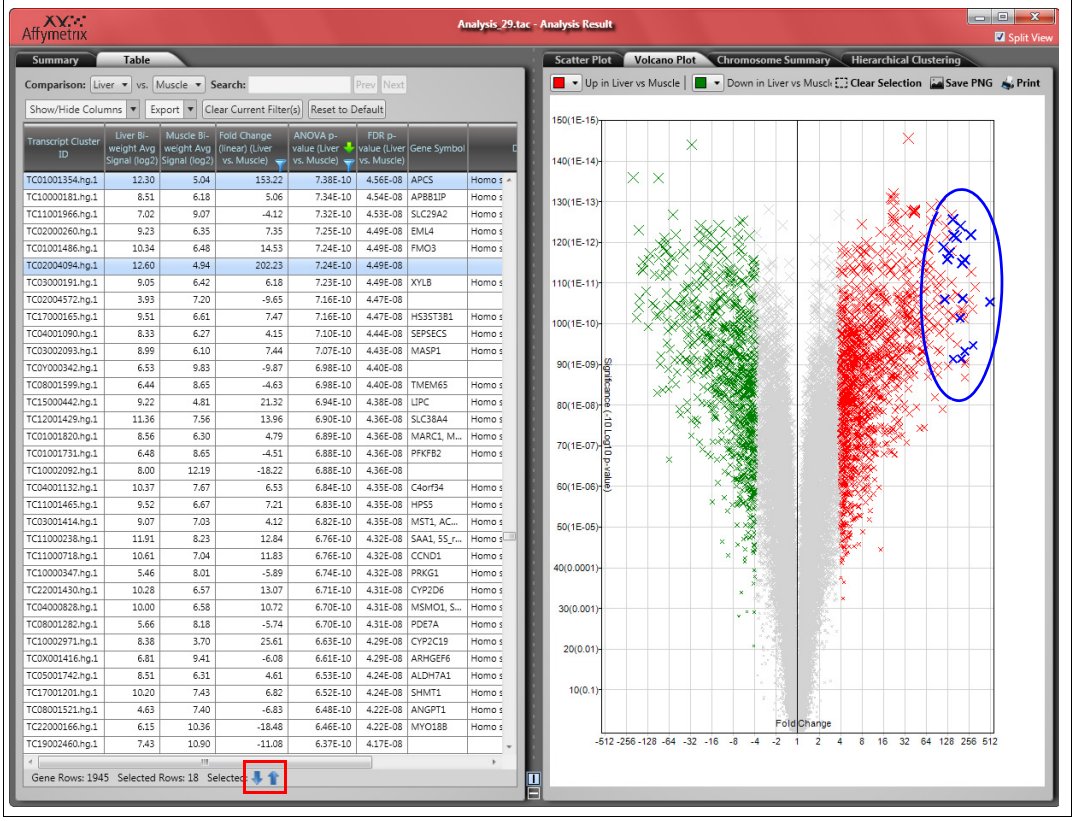
! TIP: If you have already lassoed while in the scatter plot, the volcano plot displays the same probe sets in blue, as graphs and tables are always in sync.

1. To lasso a gene or a group of genes, hold down the left mouse button and use the ***cross hair*** cursor to encircle the gene(s) of interest. (Figure 3.35)

Once a complete circle is made, the genes of interest are highlighted in blue.

The data for these genes are also highlighted (blue) inside the table view, as shown below (Figure 3.35).

Figure 3.35 Volcano Plot Graph and Table



2. Click the **blue** up or down arrows (shown) to navigate through each highlighted gene or right click in the table to get the gene symbols/IDs of these lassoed genes.

Using the Table to Investigate Lassoed Selections

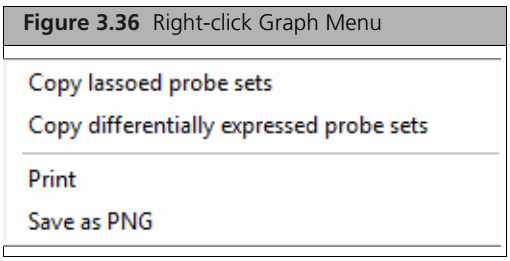
See "Saving Table Information" on page 61 to search and copy your lassoed selection.

Copying Lassoed Selections

1. Right-click on the graph.

The following menu appears. (Figure 3.36)

Figure 3.36 Right-click Graph Menu



Copy lassoed probe sets

1. Click **Copy lassoed probe sets** to copy your currently lassoed probes to the Windows Clipboard for pasting.

Copy differentially expressed probe sets

1. Click **Copy differentially expressed probe sets** to the Windows Clipboard for pasting.

Print

1. Click **Print** to print the graph to a pre-configured printer.

The Print window appears.

2. Configure the printing options as you normally would, then click **OK**.

Save as PNG

1. Click **Save PNG** to save the graph as a .PNG image file

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the graph, then click **Save**. The graph is now saved as a .**png** file.

Clearing Lassoed Selections

This option is enabled after genes have been lassoed as points of interest.

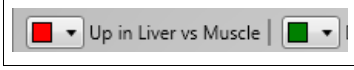
1. Click Clear Selection to clear lassoed genes from the graph and table.

TIP: You can also clear a lassoed selection by lassoing a white (blank) space within the graph.

Changing Graph Colors

Use the drop-down menus to select your up and down regulated probe set graph colors. (Figure 3.37

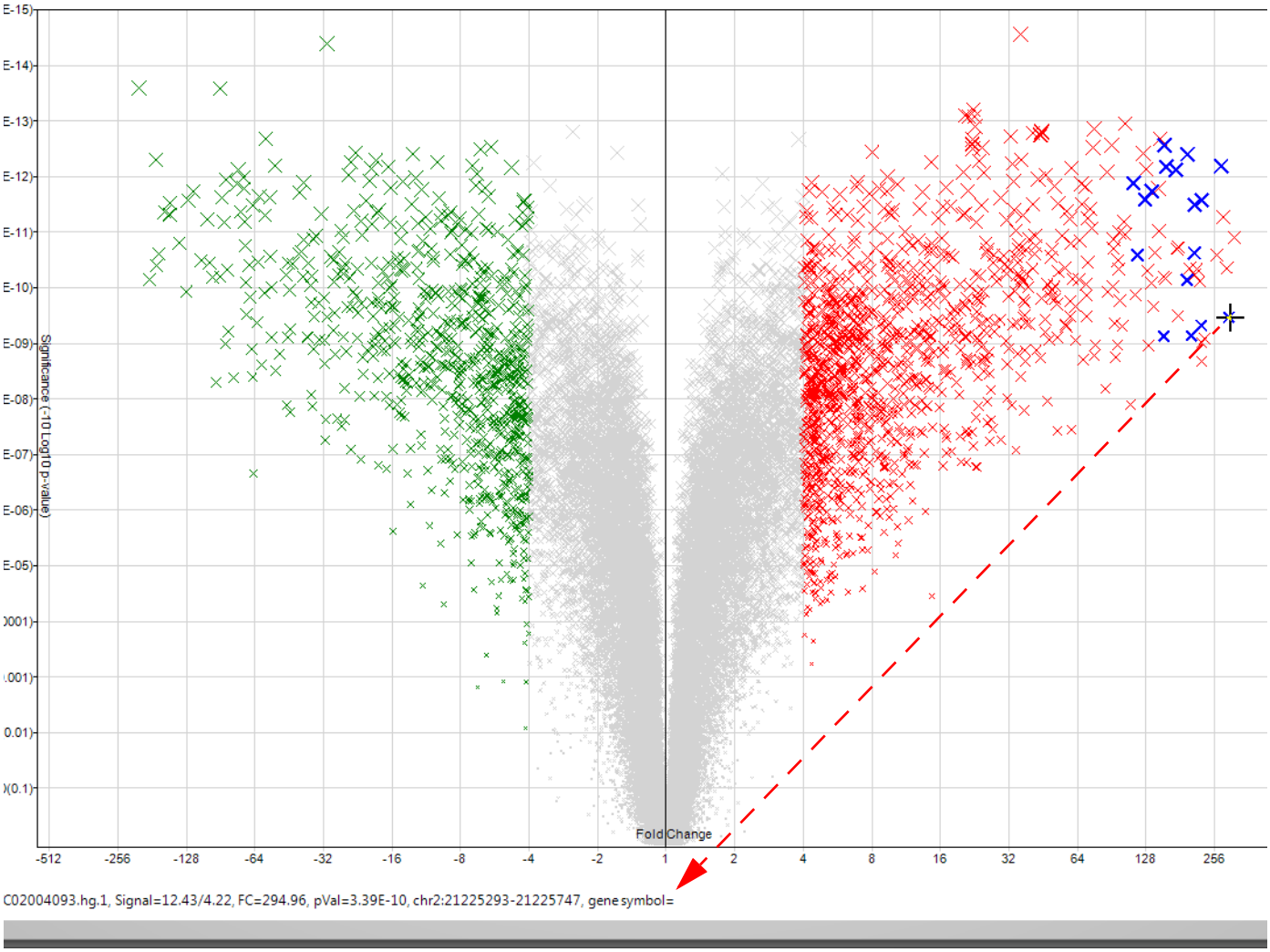
Figure 3.37 Graph Color Menus (Red and Green are default)



Obtaining Information Related to Individual Probe Sets

1. Mouse over (position the cursor over) a probe set to show its details. In the example below, (Figure 3.38) signals from both conditions (12.45 is the signal in Liver (Condition1); and 4.22 is the signal in Muscle (Condition2)) are shown, Fold change (FC) in linear scale, ANOVA p-value, chromosome positions and gene symbol are also identified in the line below. (Figure 3.38).

Figure 3.38 Mouse Over Example



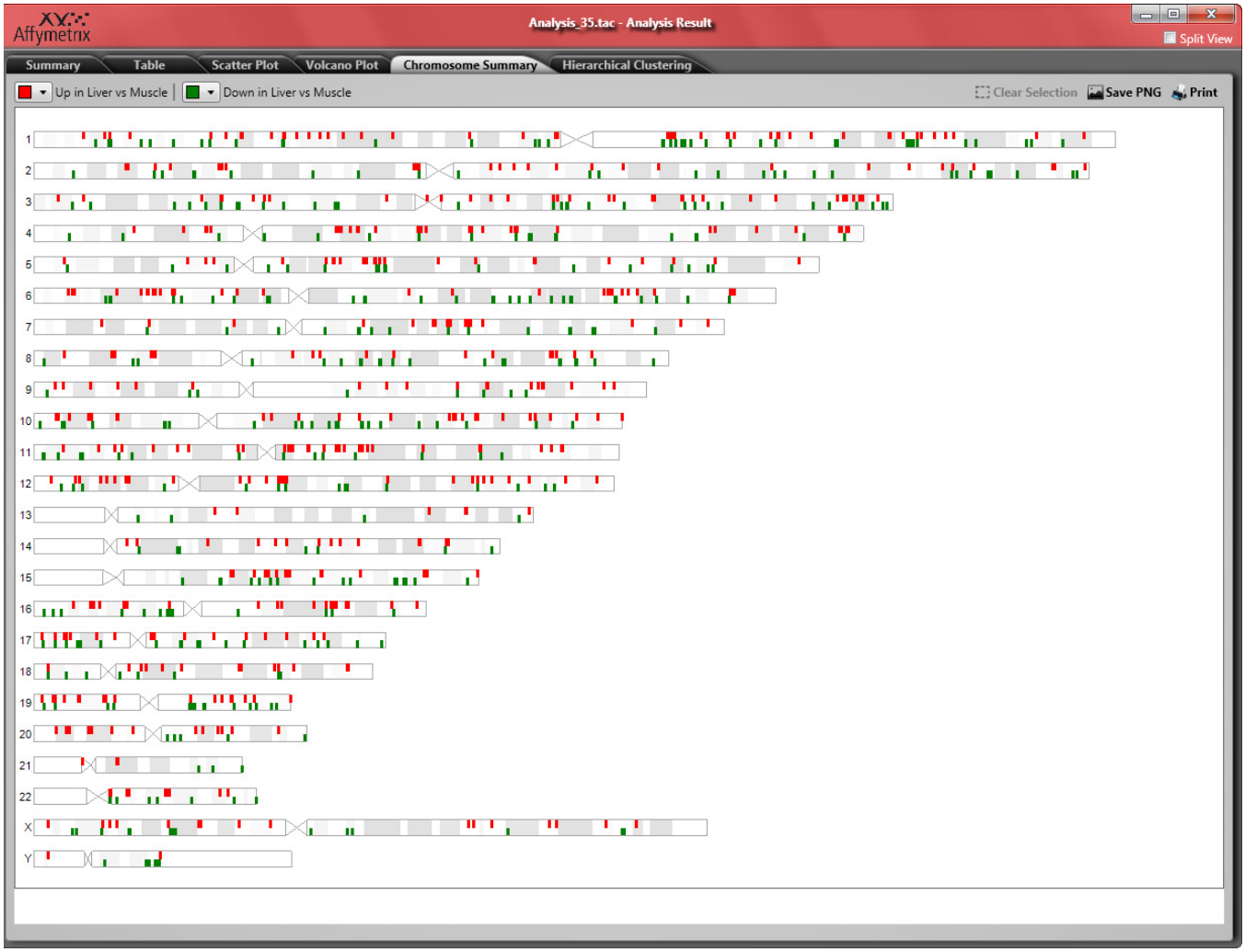
TIP: The best way to view individual probe sets is to make the graph full screen by un-checking Split View

Chromosome Summary Graph (Overview)

- The Chromosome Summary graph is a visual summary of your results on chromosomes.
- Probe Sets are plots based on their chromosomal positions, however Probe Sets without chromosomal positions are not plotted on the graph. Probe Sets with non-standard chromosome assignments are also not plotted. Keep this in mind, as you may see less probe sets in the chromosome summary graph than the scatter and volcano plots.
- In the example below (Figure 3.39), up-regulated probe sets in Liver (Condition1) vs. Muscle (Condition2) are plotted on the top of a chromosome in red. The down-regulated probe sets in Liver (Condition1) vs. Muscle (Condition2) are plotted at the bottom of a chromosome in green.

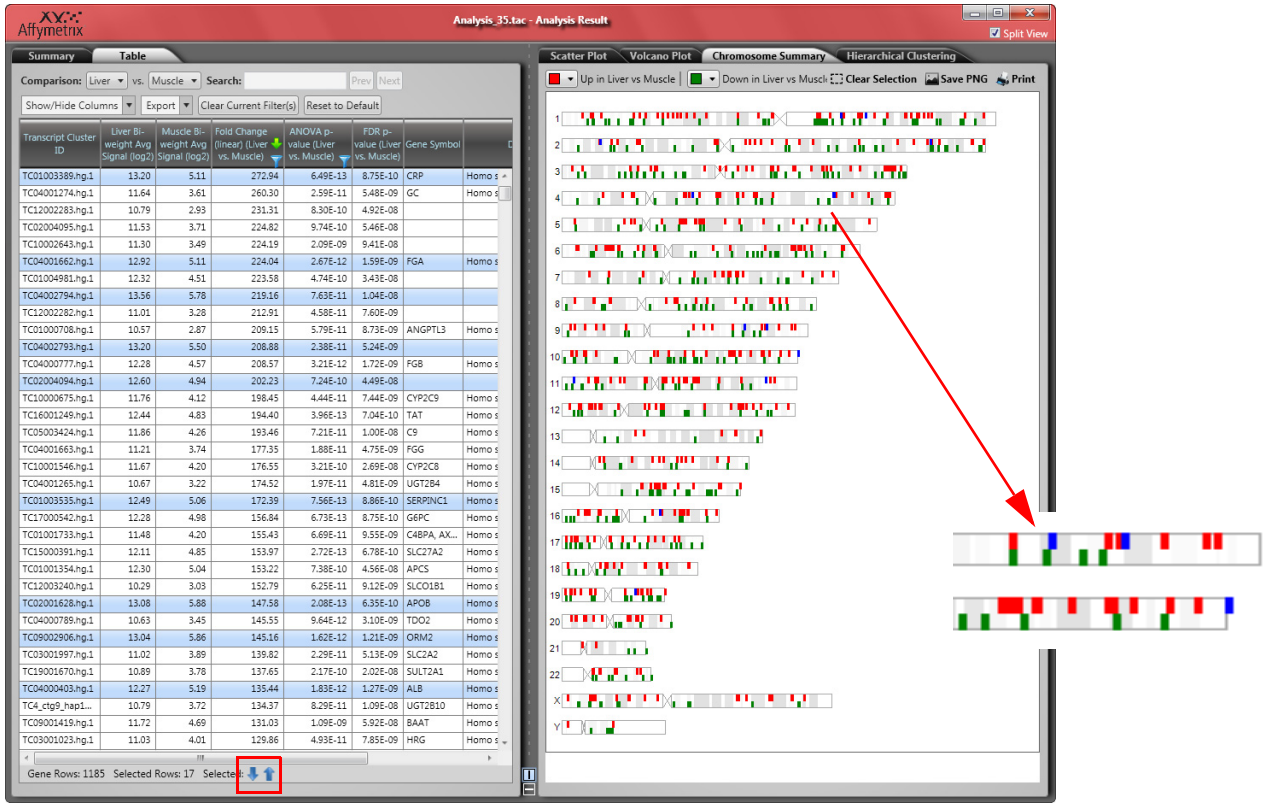
- The size of each chromosome summary square represents its gene size, therefore, the larger the square, the larger the gene. In some instances, a square appears larger, because it may contain more than one probe set (due to the minimal pixel limitation). Also, a square might contain multiple probe sets due to minimal pixel limitation.

Figure 3.39 Chromosome Summary window



Previously lassoed genes (in blue) are also reflected in the Chromosome Summary graph. (Figure 3.40)

Figure 3.40 Table and Chromosome Summary



1. Click the **blue** up or down arrows (shown) to navigate through each highlighted gene or right click in the table to get the gene symbols/IDs of these lassoed genes.

Using the Table to Investigate Pre-Lassoed Selections

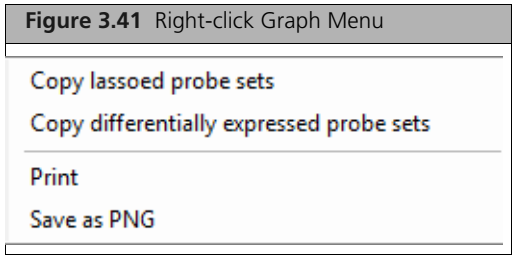
See "Saving Table Information" on page 61 to search and copy your lassoed selection.

Copying Lassoed Selections

1. Right-click on the graph.

The following menu appears. (Figure 3.41)

Figure 3.41 Right-click Graph Menu



Copy lassoed probe sets

1. Click **Copy lassoed probe sets** to copy your currently lassoed probe sets to the Windows Clipboard for pasting.

Copy differentially expressed probe sets

1. Click **Copy differentially expressed probe sets** to the Windows Clipboard for pasting.

Print

1. Click **Print** to print the graph to a pre-configured printer.

The Print window appears.

2. Configure the printing options as you normally would, then click **OK**.

Save as PNG

1. Click **Save PNG** to save the graph as a .PNG image file

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the graph, then click **Save**. The graph is now saved as a .**png** file.

Clearing Lassoed Selections

This option is enabled after genes have been lassoed.

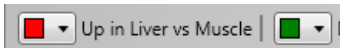
1. Click Clear Selection to clear lassoed genes from the graph and table.

TIP: You can also clear a lassoed selection by lassoing a white (blank) space within the graph.

Changing Graph Colors

Use the drop-down menus to select your up and down regulated probe set graph colors. (Figure 3.42

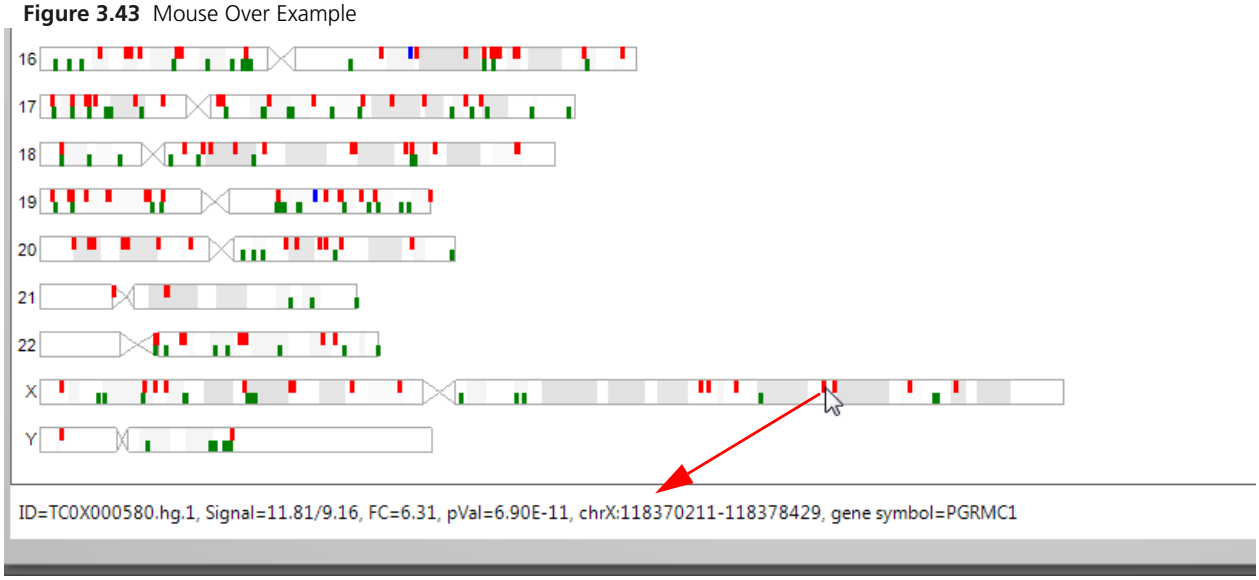
Figure 3.42 Graph Color Menus (Red and Green are default)



Obtaining Information Related to Individual Probe Sets

1. Mouse over (position the cursor over) a probe set to show its details. In the example below, (Figure 3.43) signals from both conditions (11.81 is the signal in Liver; and 9.16 is the signal in Muscle (Condition2) are shown, Fold change (FC) in linear scale, ANOVA p-value, chromosome positions and gene symbol are also identified in the line below (Figure 3.43).

Figure 3.43 Mouse Over Example



TIP: The best way to view individual probe sets is to make the chromosome summary full screen by un-checking Split View

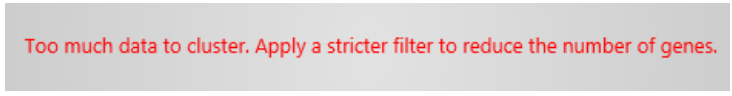
Hierarchical Clustering Graph

Hierarchical Clustering is a method of cluster analysis which seeks to build a hierarchy of clusters for use as a data mining tool.

1. Click on the **Hierarchical Clustering** tab.

If the message (Figure 3.44) appears, click on the **Table** tab to apply a stricter filter.

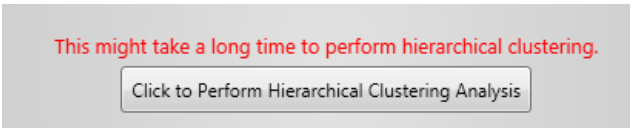
Figure 3.44 Clustering Filter Alert



For instructions on how to modify filters, **See "Filtering Column Data" on page 57**.

The following message and button appears: (Figure 3.45)

Figure 3.45 Hierarchical Clustering Analysis Button

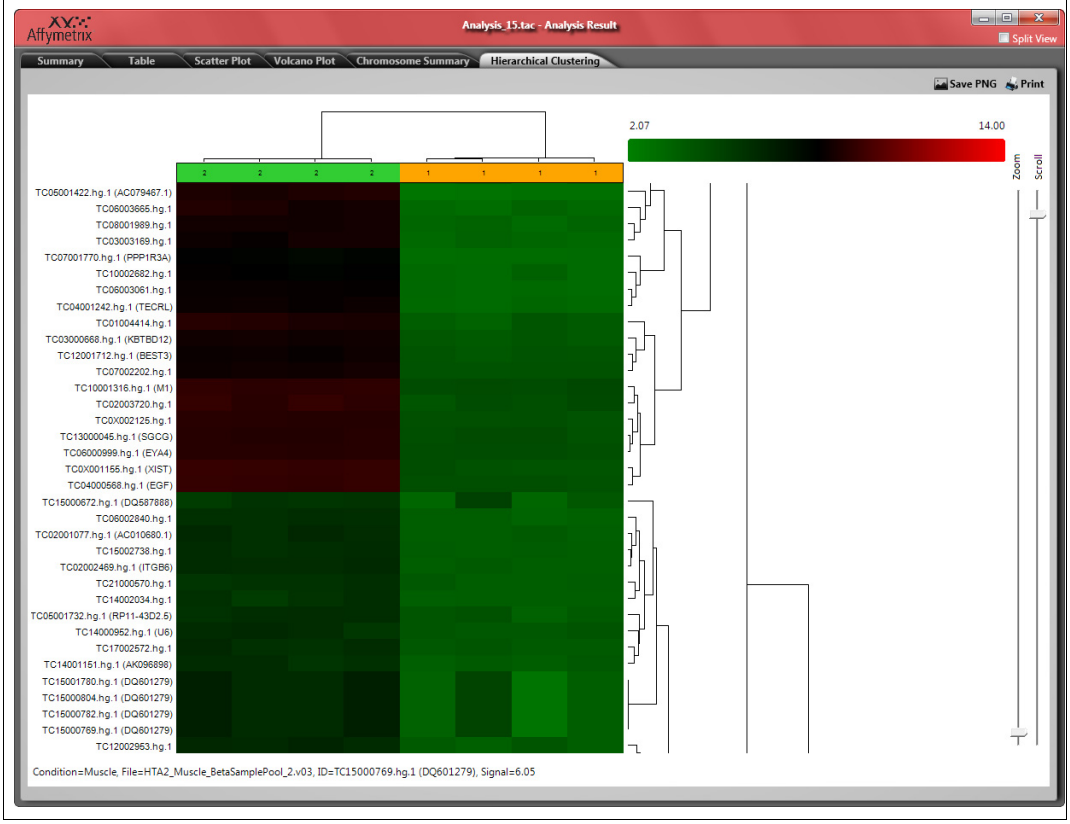


2. Click **Click to Perform Hierarchical Clustering Analysis**.

After a few moments, the Hierarchical Clustering Analysis results appear. (Figure 3.46)

NOTE: The probe sets from your current condition pair that pass the filter criteria are sent to clustering. The results shown reflect your current condition pairings only. To confirm what pairing is active, refer to the TAC Table's Comparison drop-down selections. (**Figure 3.22**)

Figure 3.46 Hierarchical Cluster - Full screen



IMPORTANT: The processing limit of the Hierarchical Clustering Graph is 5000 genes.

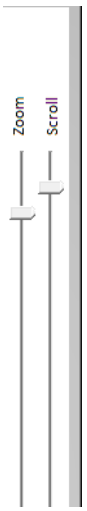
Selecting Interesting Probe Sets

There are 3 methods to select interesting probe sets.

Method 1: Zoom Slider

1. Click on the **Zoom** slider bar (Figure 3.47), then hold down the left mouse button and move the bar down to magnify point(s) of interest

Figure 3.47 Zoom and Scroll Tool



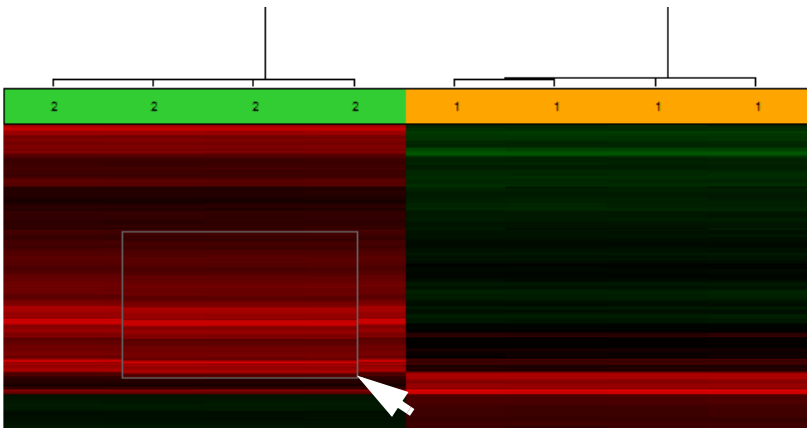
Method 2: Scroll Slider

1. Click on the **Scroll** slider bar (Figure 3.47), then hold down the left mouse button and move the bar up or down to scroll through the results.

Method 3: Mouse Zoom

1. To zoom in on a region of interest, left-click, hold, then move the mouse to frame your selection. (Figure 3.48)

Figure 3.48 Mouse Zoom Tool

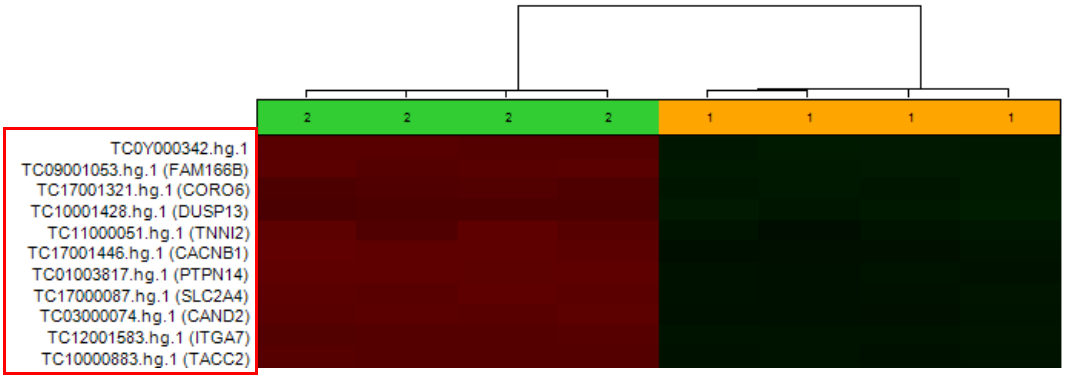


2. Release the mouse button.

Your region of interest is now magnified revealing its Transcript Cluster IDs and their Gene Symbols (left column). (Figure 3.49) You can also right-click on a probe set of interest, then export it.

- To Export Probe Sets, (**See "Exporting Probe Sets of Interest" on page 78**

Figure 3.49 Mouse Zoom Tool Result

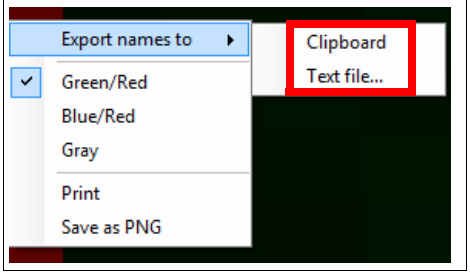


Exporting Probe Sets of Interest

1. Right-click on the probe sets that are shown in the current window

The following menu appears: (Figure 3.50)

Figure 3.50 Right-Click Mouse Menu



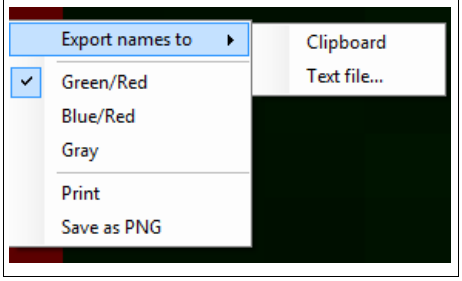
- Export names to
  Clipboard - Copies your export data to the Windows Clipboard for pasting.
  Text file... - Saves your export data as a .txt file.

Changing Graph Colors

1. Right-click on the graph.

The following menu appears: (Figure 3.51

Figure 3.51 Right-Click Mouse Menu



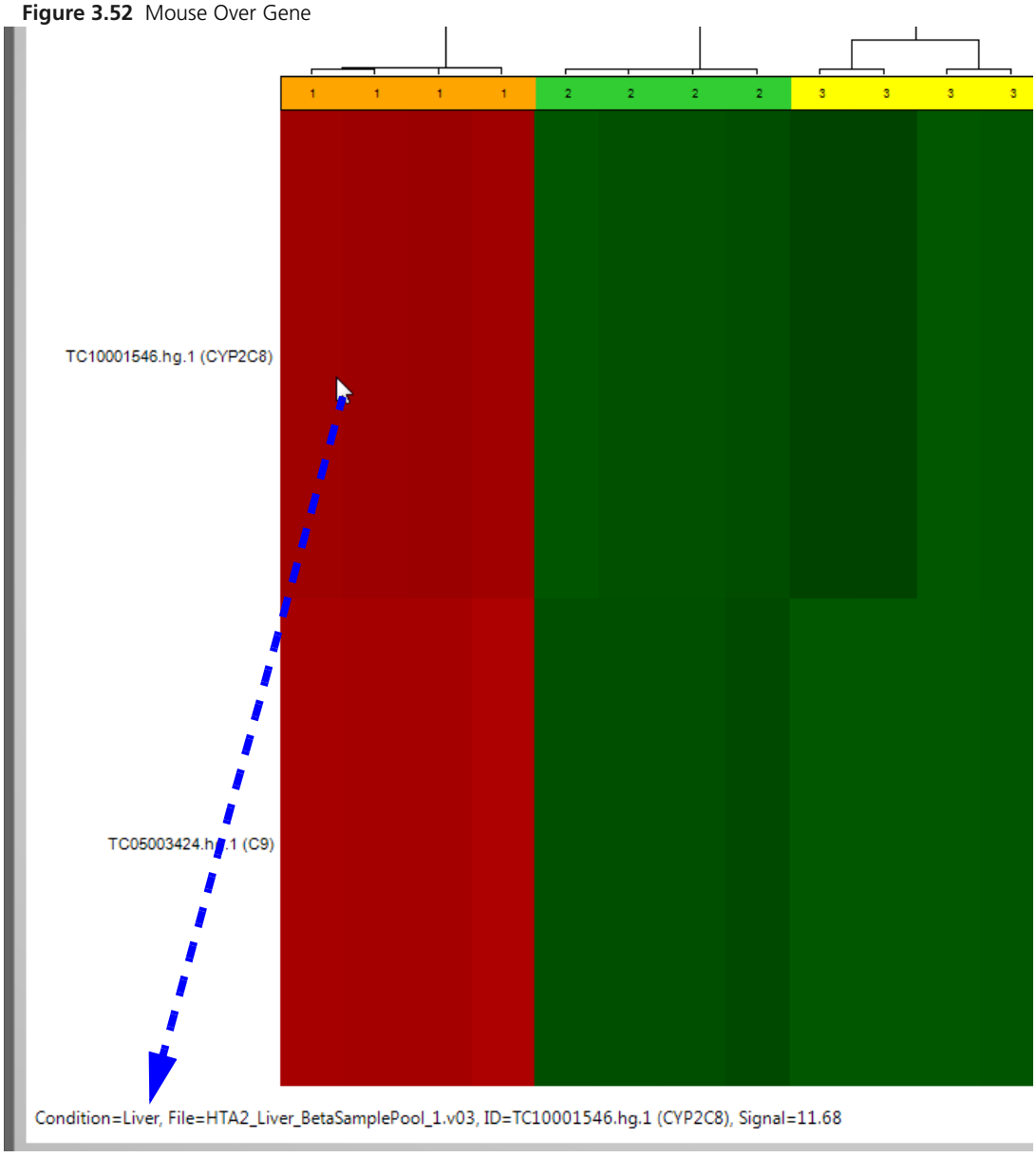
2. Click to select a desired color combination.

- Green/Red - Colors range from green to red.
- Blue/Red - Colors range from blue to red.
- Gray - Tones range from black to light gray.

Obtaining Information Related to Individual Probe Sets

1. Mouse over (position the cursor over) a probe set to show its details. In the example below, the signal from one of the Liver samples is shown as 11.68, as well as the ID, Filename, and Condition name. (Figure 3.52).

Figure 3.52 Mouse Over Gene



Conditions

This header displays the Conditions. (Figure 3.53)

1. Mouse over a probe set (below the condition legend) to show what number represents what condition.

Figure 3.53 Condition Legend

Signal Intensity Scale

Displays the signal intensity range of your current condition pair from minimum to maximum. (Figure 3.54)

Figure 3.54 Signal Intensity Scale

Saving a Hierarchical Cluster

1. To save the cluster as a .PNG image file, click **Save PNG**.

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the cluster, then click **Save**. The cluster is now saved as a **.png** file.

Printing Option

1. To print the cluster to a pre-configured printer, click **Print**.

The Print window appears.

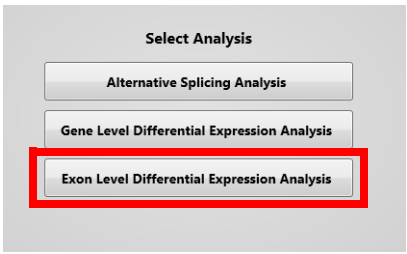
2. Configure the printing options as you normally would, then click **OK**.

Exon Level Differential Expression Analysis

Setting Up an Analysis Using Exon CHP Files

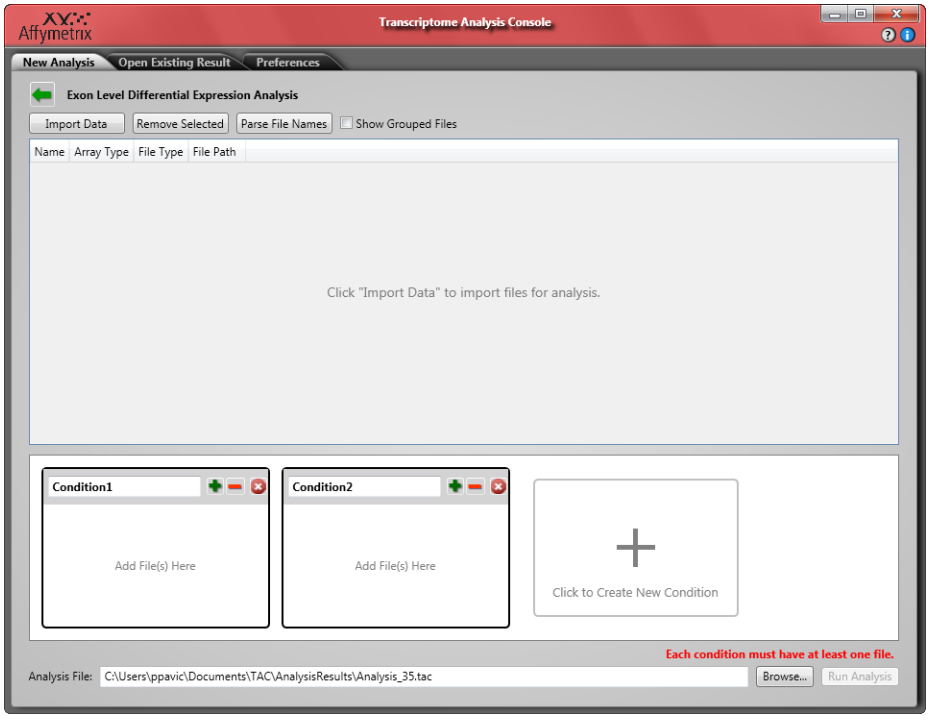
1. At the main TAC window, click **Exon Level Differential Expression Analysis**. (Figure 4.1

Figure 4.1 Main TAC window



The New Analysis window appears. (Figure 4.2)

Figure 4.2 New Analysis window

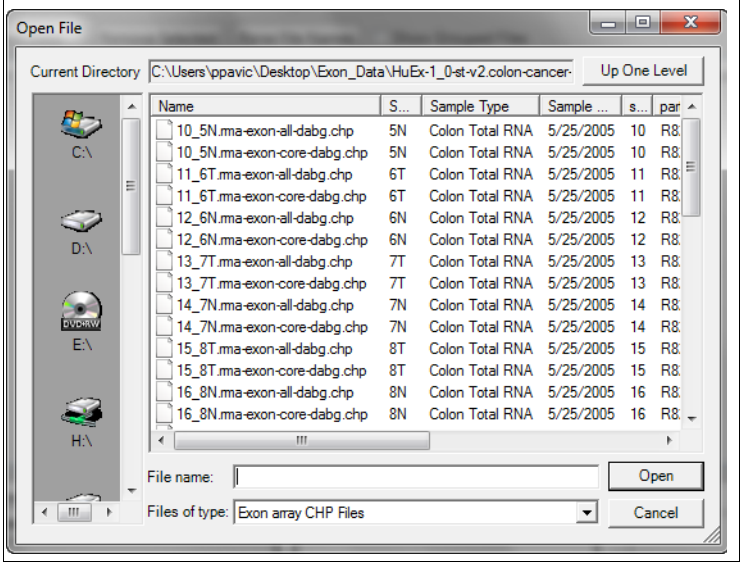


2. Click **Import Data**.

The following window appears. (Figure 4.3) It displays the data path you set up earlier and its files.

NOTE: The first time you launch TAC, it asks you to define a path to store your library and annotation files. For your convenience, TAC retains this path information. Affymetrix recommends you use the Expression Console library path you already configured.

Figure 4.3 Import Data Dialog window



3. Single click, Ctrl click, or Shift click to select multiple files (as shown above).

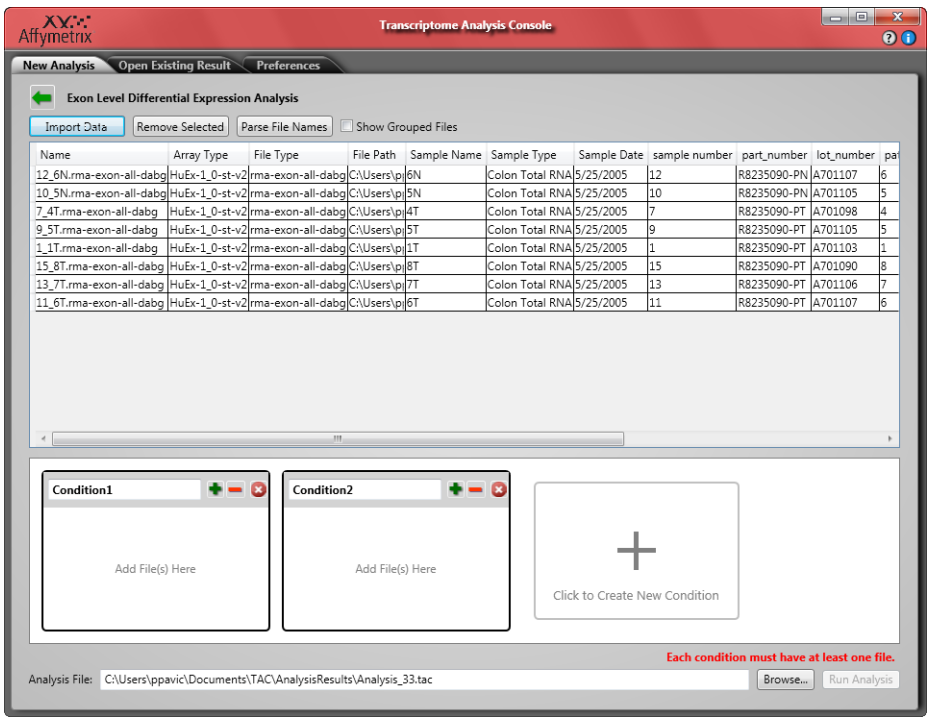
IMPORTANT: You MUST only import 1 type of file into your conditions. In the example below, "ma-exon-all.dabg files were selected.

NOTE: To optimize the analysis, Affymetrix recommends importing more than 1 sample per condition.

4. Click Open.

The selected files are now populated in the Sample File Window. (Figure 4.4)

Figure 4.4 Import Data into Sample File Window



- Click Show Grouped Files checkbox (Figure 4.4) to display sample file names and attributes even they have been added to various conditions. Samples that have been added to different conditions get grayed out.
- Click Remove Selected (Figure 4.4) to remove a file(s) from the Sample File window.

Parsing Imported Data File Names (Optional)

This option gives you the ability to parse attributes from the sample file names and helps you set up conditions. **See "Parsing Imported Data File Names (Optional)" on page 49.**

Importing CHP Files into Different Condition Groups

! IMPORTANT: Customize your condition names first, then add the CHP files into each condition.

1. Click on the **Condition1** window header field to rename it to an appropriate Condition name. (Figure 4.5)
2. Click to select and highlight the data you want to use for Condition1.
3. Click ✚ in the Condition1 window to add your selected files to the Condition1 window.
4. If needed, click ▬ to move selected files back to the Sample File window.
5. If needed, click ⊗ to delete your current Condition and move all its files back to the Sample File window.
6. Repeat the steps 1-3 (above) for Condition2.
7. To create more than 2 conditions, click **Click to Create New Condition** (Figure 4.5), then repeat steps 1-3 (above) for your 3rd Condition.

8. If needed, edit your Analysis result file path and/or name by clicking inside the **Analysis File** text field (Figure 4.6), or click **Browse** to select a new file destination.

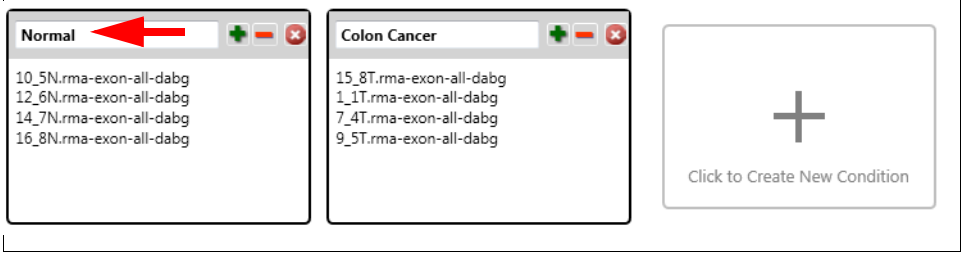
Importing Files using Drag and Drop

1. Click Shift or click Ctrl, to select a group of files.
2. Click and hold onto the last file in the group, then drag them into the appropriately labeled Condition window.
3. Release the mouse button.

The Condition1 (Normal) window now contains your files.

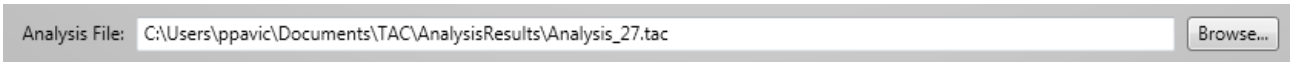
4. If needed, click ➖ to move a selected file back to the Sample File window.
5. If needed, click ⛔ to delete your current Condition name and move all its files back to the Sample File window.

Figure 4.5 Edited Condition1 name with samples



6. Repeat the steps 1-3 (above) for Condition2 (Colon Cancer)
7. To create more than 2 conditions, click **Click to Create New Condition** (Figure 4.5), then repeat steps 1-3 (above) for your 3rd Condition.
8. If needed, edit your Analysis result file path and/or name by clicking inside the **Analysis File** text field (Figure 4.6), or click **Browse** to select a new file destination.

Figure 4.6 Editable Analysis Result File Paths

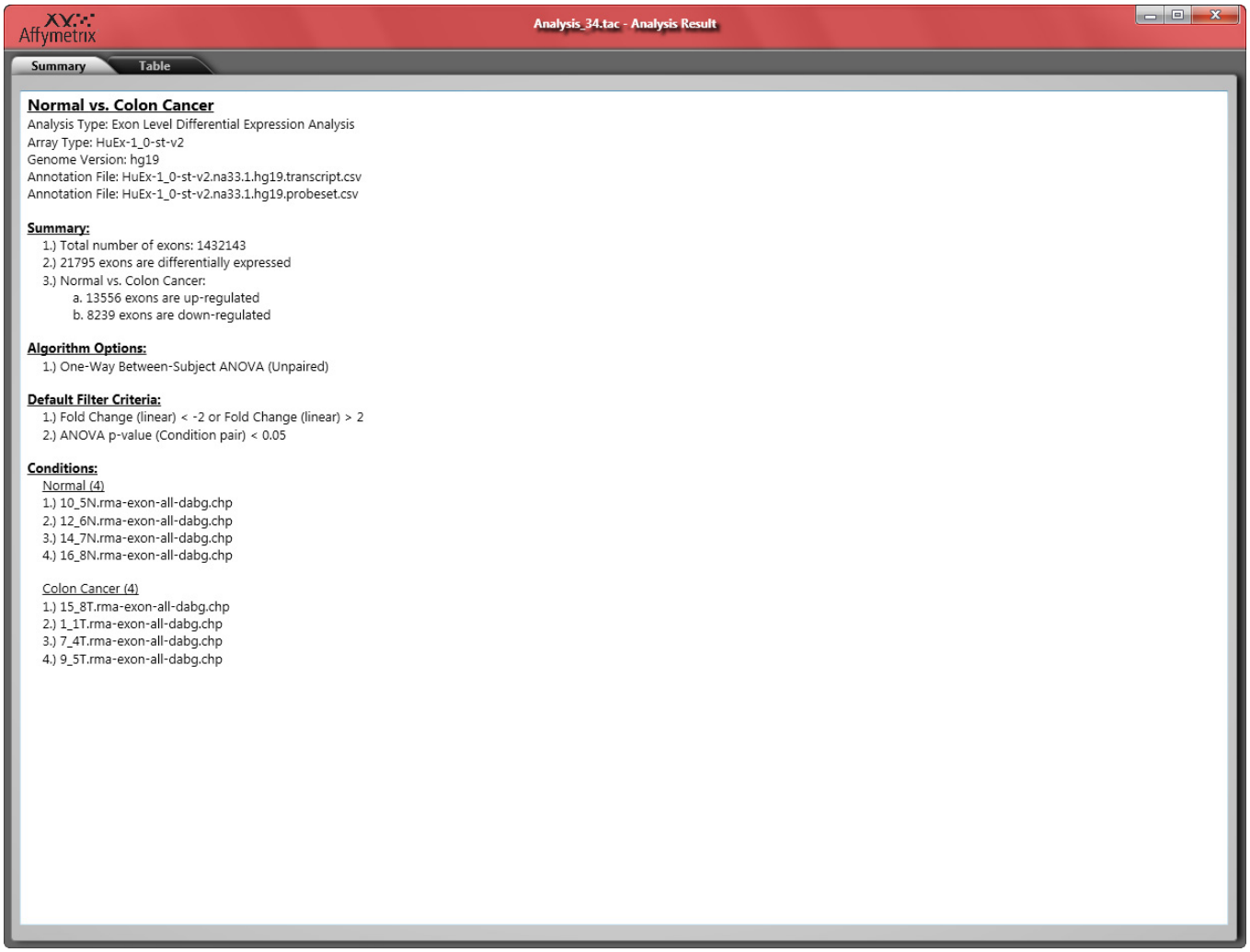


9. After the Conditions have been labeled and populated, click Run Analysis.

! TIP: TAC auto-saves your studies. At any time, click on the Open Existing Result tab to view recent analysis results.

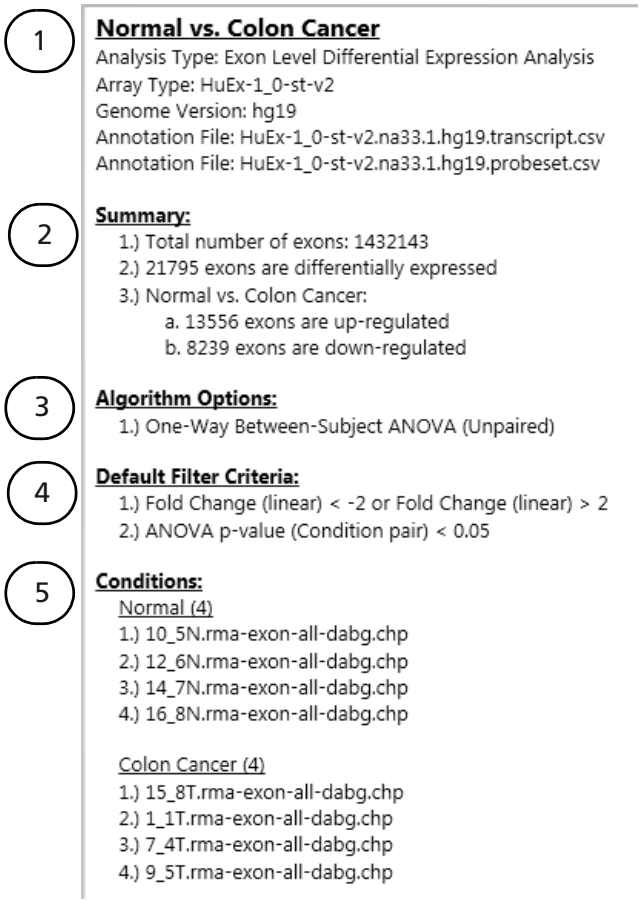
Please Wait... appears. then the Analysis Result viewer appears. By default, the Summary tab appears. (Figure 4.7).

Figure 4.7 Default view after running an analysis



Exon Level Differential Expression Analysis Summary Window (Overview)

The Summary information is static and based on the algorithm parameters applied during the analysis. Below is an example of the information collected in the Summary window:



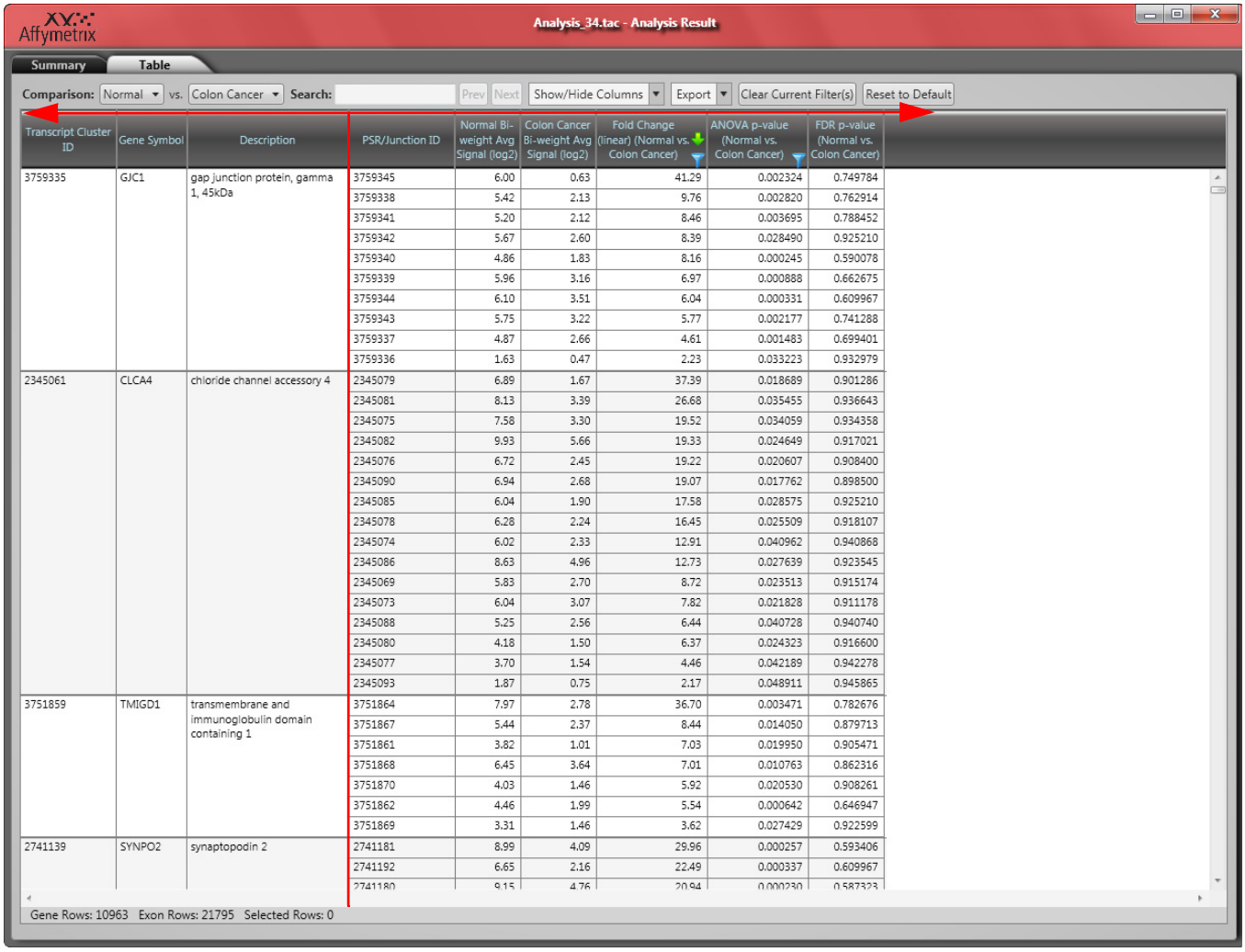
1. Summary information per this analysis. NOTE: Summaries vary between Gene, Exon, and Splicing analysis.
2. Array summary. Total number of Exons, number of Exons that are differentially expressed, and the number of Exons that are up and down regulated.
3. Shows the algorithm used to perform the Exon Level Differential Expression Analysis.
4. This section displays the factory default filtering criteria results. NOTE: Only Exons that pass in the Exon table criteria are summarized.
5. Displays each Condition name and the total number of CHP files in it.

Exon Level Differential Expression Analysis Table Window (Overview)

1. After reviewing the Summary, click the **Table** tab to display the Table in full screen. (Figure 4.8)

! NOTE: The left side of the table provides gene level information. The right side of the table provides exon information and is organized by each transcript cluster. (Figure 4.8)

Figure 4.8 Exon Table



Transcript Cluster ID	Gene Symbol	Description	PSR/Junction ID	Normal Bi-weight Avg Signal (log2)	Colon Cancer Bi-weight Avg Signal (log2)	Fold Change (linear) (Normal vs. Colon Cancer)	ANOVA p-value (Normal vs. Colon Cancer)	FDR p-value (Normal vs. Colon Cancer)
3759335	GJC1	gap junction protein, gamma 1, 45kDa	3759345	6.00	0.63	41.29	0.002324	0.749784
			3759338	5.42	2.13	9.76	0.002820	0.762914
			3759341	5.20	2.12	8.46	0.003695	0.788452
			3759342	5.67	2.60	8.39	0.028490	0.925210
			3759340	4.86	1.83	8.16	0.000245	0.590078
			3759339	5.96	3.16	6.97	0.000888	0.662675
			3759344	6.10	3.51	6.04	0.000331	0.609967
			3759343	5.75	3.22	5.77	0.002177	0.741288
			3759337	4.87	2.66	4.61	0.001483	0.699401
			3759336	1.63	0.47	2.23	0.033223	0.932979
2345061	CLCA4	chloride channel accessory 4	2345079	6.89	1.67	37.39	0.018689	0.901286
			2345081	8.13	3.39	26.68	0.035455	0.936643
			2345075	7.58	3.30	19.52	0.034059	0.934358
			2345082	9.93	5.66	19.33	0.024649	0.917021
			2345076	6.72	2.45	19.22	0.020607	0.908400
			2345090	6.94	2.68	19.07	0.017762	0.898500
			2345085	6.04	1.90	17.58	0.028575	0.925210
			2345078	6.28	2.24	16.45	0.025509	0.918107
			2345074	6.02	2.33	12.91	0.040962	0.940868
			2345086	8.63	4.96	12.73	0.027639	0.923545
			2345069	5.83	2.70	8.72	0.023513	0.915174
			2345073	6.04	3.07	7.82	0.021828	0.911178
			2345088	5.25	2.56	6.44	0.040728	0.940740
			2345080	4.18	1.50	6.37	0.024323	0.916600
			2345077	3.70	1.54	4.46	0.042189	0.942278
			2345093	1.87	0.75	2.17	0.048911	0.945865
3751859	TMIGD1	transmembrane and immunoglobulin domain containing 1	3751864	7.97	2.78	36.70	0.003471	0.782676
			3751867	5.44	2.37	8.44	0.014050	0.879713
			3751861	3.82	1.01	7.03	0.019950	0.905471
			3751868	6.45	3.64	7.01	0.010763	0.862316
			3751870	4.03	1.46	5.92	0.020530	0.908261
			3751862	4.46	1.99	5.54	0.000642	0.646947
			3751869	3.31	1.46	3.62	0.027429	0.922599
2741139	SYNPO2	synaptopodin 2	2741181	8.99	4.09	29.96	0.000257	0.593406
			2741192	6.65	2.16	22.49	0.000337	0.609967
			2741180	9.15	4.76	20.94	0.000230	0.587323

Parts of the Table

- Column Headers
- Table Options

Column Headers

The factory default columns and 2 preset filters are as shown: (Figure 4.9)

See Table 4.1 for definitions of these columns.

Figure 4.9 Default Table Column Headers

Showing or Hiding Table Columns

1. Click the Show/Hide Columns drop-down menu to show or hide columns in the Exon table.
2. Click outside the Show/Hide Drop-down menu to close it.

Table 4.1 Exon Table Columns and their Definitions (Column entries in BOLD are factory defaults)

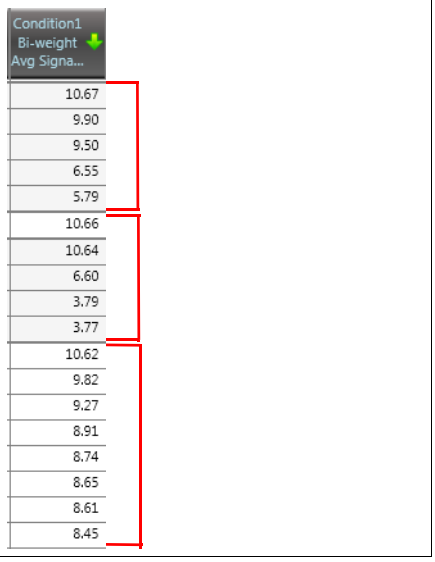
Available Columns	Description
Transcript Cluster ID	ID of Transcript Cluster (TC)
Gene Symbol	Gene symbol for this transcript cluster. Note: RefSeq gene symbol is listed as the first gene symbol (if there are more than 1 gene symbol). Note: A TC with no gene symbol, may be auto-assigned a public gene ID.
Description	Gene Description for this TC.
Chromosome	Chromosome for this transcript cluster. See Chromosome Naming Scheme[3] for a detailed description.
Genomic Position	Genomic Start/Stop position for this TC.
Public Gene IDs	Public Gene IDs for this TC.
PSR/Junction ID	ID of Probe Selection Region (PSR) and Junction Probe Sets.
(Condition1) Bi-weight Avg Signal (log2) **(Condition2) Bi-weight Avg Signal (log2)**	Tukey's Bi-weight[1] average of exon intensity of all the samples in a condition: Bi-weight average of (sample 1 exon1 intensity + sample 2 exon1 intensity +…+ sample N exon1 intensity.
(Condition1) Standard Deviation (Condition2) Standard Deviation	Standard Deviation[2] of exon intensities from all samples in a condition: STDEV of (sample 1 exon1 intensity + sample 2 exon1 intensity +…+ sample N exon1 intensity)
Fold Change (linear) (Condition1 vs. Condition2)	This shows the fold change (in linear space) of Condition1 vs. Condition2. $2^{[\text{Condition1 Bi-weight Avg Signal (log2) - Condition2 Bi-weight Avg Signal (log2)}]}$
ANOVA p-value (Condition1 vs. Condition2)	One-Way Between-Subject ANOVA[2] p-value (Condition 1 vs. Condition2)
FDR p-value (Condition1 vs. Condition2)	FDR adjusted p-value based on Benjamini-Hochberg Step-Up FDR-controlling Procedure[5] IMPORTANT: All ANOVA p-values from all exons are sent to FDR for correction.

Sorting Columns

TAC uses a 2-level sorting process for the exon information (right side of the Exon table).

First, it sorts exon within each gene based on the exon data, then it sorts each gene. (Figure 4.10)

Figure 4.10 2-Level Sort Results (Example)

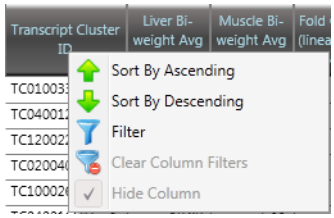


Right-Click Method

1. Select a column, then right-click on it.

The following window appears: (Figure 4.11)

Figure 4.11 Right-click Column Menu



2. Click to select either **Sort By Ascending** or **Sort By Descending**.

Double-Click Method

1. Double-click on a column header to sort its data in an ascending order. Double-click on the same column header to sort its data in a descending order.

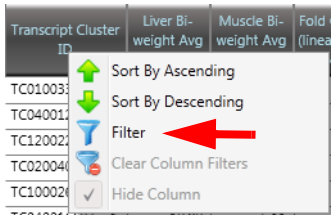
Filtering Column Data

All table columns are filterable.

1. Select a column, then right-click on it.

The following window appears: (Figure 4.11)

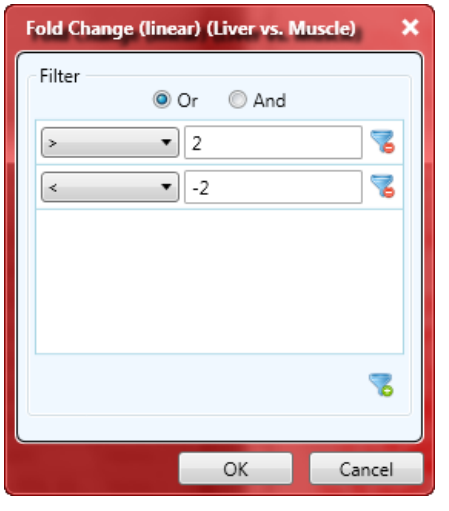
Figure 4.12 Right-click Column Menu



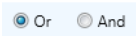
2. Click **Filter**.

The following window appears (Fold Change column example shown): (Figure 4.13)

Figure 4.13 Filter Properties



Editing Filtering Properties:

1. Click the **Or** or **And** button to choose **Or** or **AND** logic. 

2. Click the symbol drop-down menu(s) to select new symbol(s).(Figure 4.14)

Figure 4.14 Drop-down Menu

3. Click inside the numbering field(s)  to enter new value(s).

4. Click  to add filter(s).
5. Click  to remove filter(s).

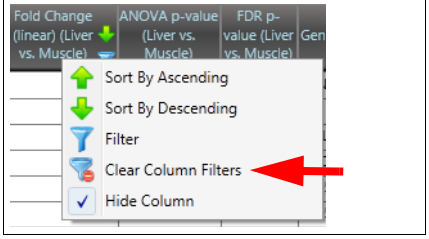
Clearing Filters

Individual Filter

1. Right-click on the filtered column you want to clear.

The following window appears: (Figure 4.15)

Figure 4.15 Right-click Column Menu



2. Click **Clear Column Filters**.

The filter is removed.

All Filters

1. Click Clear Current Filter(s) remove all currently active filters from the Splicing Table.

Table Options

Use the Table Options Menu (Figure 4.16) to customize your table view.

Figure 4.16 Table options menu

Rearranging Column Orders

1. Click on a column you want to move.
2. Drag it (left or right) to its new location.
3. Release the mouse button.

The column is now in its new position.

Resetting Table Defaults

1. Click Reset to Default to return the table to its factory setting.

Searching Keywords

! NOTE: The Search Tool is limited to finding matching strings. It is not a full search engine.

1. To search for a keyword within your table, click inside the **Search** field, then type your keyword.
2. Click the Prev or Next buttons to search. (Figure 4.17)

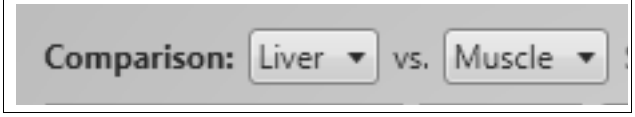
Figure 4.17 Search Tool

Changing Condition vs. Condition Pairings

1. Use the **Comparison** drop-down menus to change your condition pairings. (Figure 4.18)

You **must** choose 2 different conditions. Identical condition pairings generates the error message, ***Please Choose Two Different Conditions***

Figure 4.18 Condition Pairing Drop-down



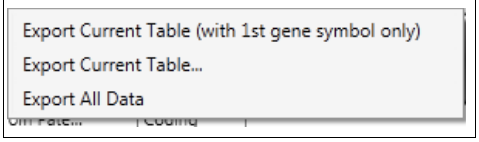
IMPORTANT: Table and graph results ONLY reflect your current Condition pairing.

Exporting Options

If you want to export (Save) your analysis table, click Export drop-down.

The following Export options appear: (Figure 4.19)

Figure 4.19 Export Menu



Exporting the Current Table with 1st Gene Symbols

NOTE: This option shows the first gene symbol only if there is more than one gene symbol for the selected transcript cluster.

1. Click **Export Current Table (with 1st gene symbol only)**.

The **Save As** window appears.

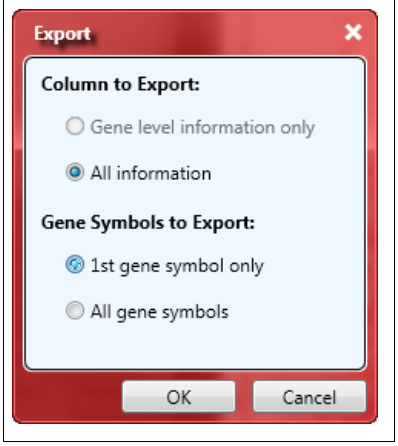
2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting the Current Table

1. Click **Export Current Table**.

The following window appears: (Figure 4.20)

Figure 4.20 Export Window



Column to Export

1. Click either **Gene level information only** or **All information**.

Gene Symbols to Export

1. Click either **1st gene symbol** or **All gene symbols**
2. Click **OK**

The **Save As** window appears.

3. Click on an existing folder or click **New Folder** to choose a new save location.
4. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

Exporting All Data

1. Click **Export All Data**.

! NOTE: Only currently paired data is exported, including data in the hidden columns, and the paired data's gene level information.

The **Save As** window appears.

2. Click on an existing folder or click **New Folder** to choose a new save location.
3. Type a filename for the table, then click **Save**. The table is now saved as a .txt file.

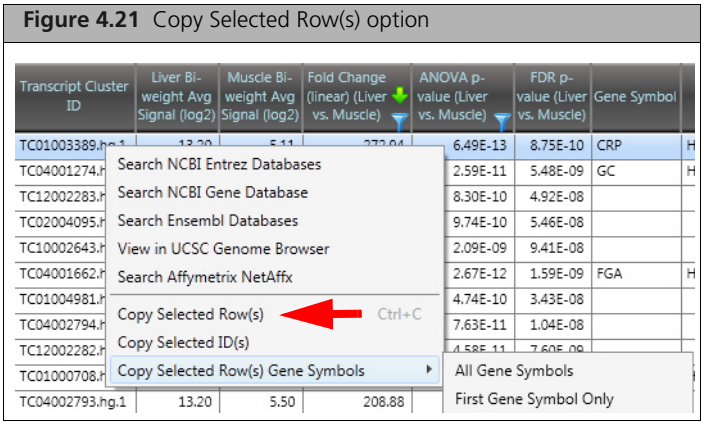
Saving Table Information

Use this copy feature to save table information to the Windows Clipboard, then use this buffered information for pasting into other applications or websites.

Copy Selected Row(s)

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.
2. Right-click, then click to select **Copy Selected Row(s)**. (Figure 4.21)

Figure 4.21 Copy Selected Row(s) option



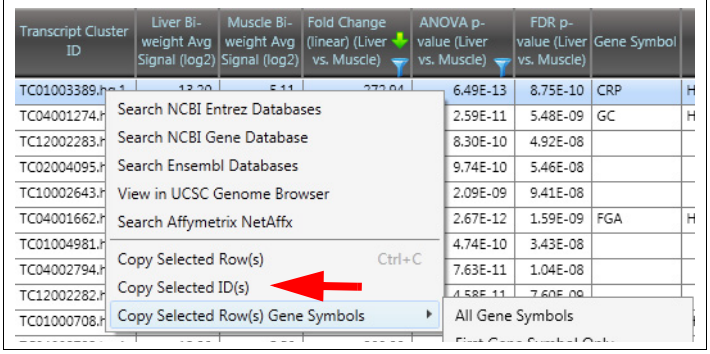
The selected gene level information (shown on the left side of the exon table) are now copied to the Windows Clipboard for pasting.

Copy Selected ID(s)

1. Click to highlight (light blue) a ID or Ctrl left-click to highlight multiple rows.

2. Right-click, then click **Copy Selected ID(s)** to copy Transcript Cluster IDs). (Figure 4.22)

Figure 4.22 Copy Selected ID(s) option

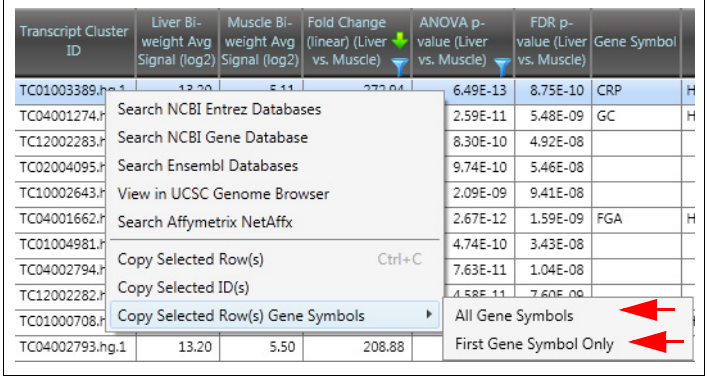


The selected TC IDs are now copied to the Windows Clipboard for pasting.

Copy Selected Row(s) Gene Symbols

1. Click to highlight (light blue) a row or **Ctrl** left-click to highlight multiple rows.
2. Right-click on the selection, then click to select **Copy Selected Row(s) Gene Symbols**.
3. Click to select either **All Gene Symbols** (all possible gene symbols for a Transcript Cluster) or **First Gene Symbol Only** (the first gene symbol that belongs to the Transcript Cluster). (Figure 4.23)

Figure 4.23 Copy Selected Row(s) gene Symbols options



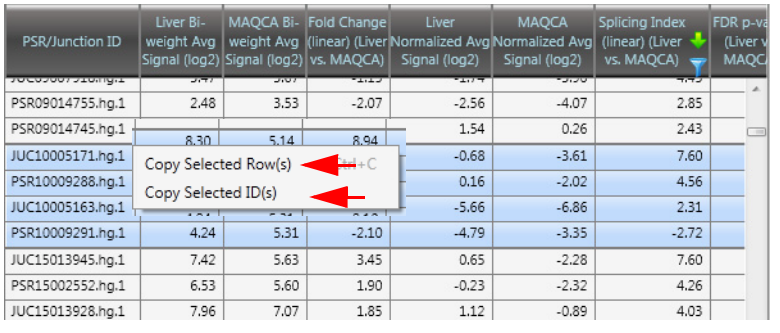
Your selected rows with gene symbols are now copied to the Windows Clipboard.

Copy Exon Information

1. Click to highlight (light blue) a exon or Ctrl left-click to highlight multiple rows.

2. Right-click, then click either **Copy Selected ID(s)** or **Copy Selected Row(s)** to copy exons. (Figure 4.24)

Figure 4.24 Copy Selected PSR/JUC option



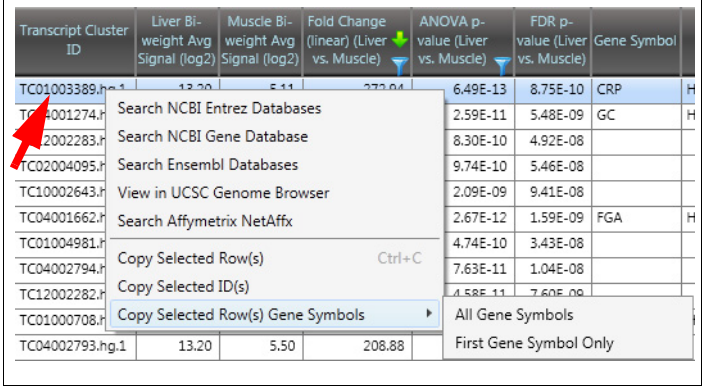
The selected exons are now copied to the Windows Clipboard for pasting.

Accessing External Databases (Internet Connection Required)

1. To link out to various external databases, right-click on a TC of interest.

The following menu appears: (Figure 4.25

Figure 4.25 Search Database menu



2. Click to select the external database you want to visit.

Your internet browser opens to the appropriate website.

Searching the Affymetrix NetAffx Website

1. Click to select **Search Affymetrix NetAffx**.

The internet browser opens to the Affymetrix Customer Login window.

2. Enter your NetAffx **Email ID** and **Password**, then click **Submit**.

Your internet browser opens to the ***Netaffx Query Center*** and displays information about your gene of interest.

! NOTE: The NetAffx Query Center is compatible with Windows Internet Explorer and Firefox. Chrome is not supported at this time.

! NOTE: If a Probe Set or Transcript Cluster is not available, an appropriate message appears.

Algorithms

1) Tukey's Bi-weight Average Algorithm

Tukey's Bi-weight average is a method to determine a robust average unaffected by outliers.

- **Step 1:** The median is determined to define the center of the data.
- **Step 2:** The distance of each data point from the median is determined. This distance is then used to determine how much each value should contribute to the average. For example, outliers that are far away from the median should contribute less to the average.
- All end result values represented as a Bi-weight average, are shown in a log2 scale (if the CHP files are summarized using RMA)
- For more information, go to:

http://media.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf

2) ANOVA and Standard Deviation are Calculated using NMATH Package

Analysis of Variance (ANOVA)

- TAC computes and summarizes a traditional unpaired One-Way (single factor) Analysis of Variance (ANOVA) for each pair of condition groups and for all condition groups (if > 2 conditions).

3) Chromosome Naming Scheme

- In addition to the “regular” chromosomes, the hg19 browser contains nine haplotype chromosomes and 59 unplaced contigs. If an unplaced contig is localized to a chromosome, the contig name is appended to the regular chromosome name, as in chr1_gl000191_random. If the chromosome is unknown, the contig is represented with the name "chrUn" followed by the contig identifier, as in chrUn_gl000211. Note that the chrUn contigs are no longer placed in a single, artificial chromosome as they have been in previous UCSC assemblies. See the sequences page for a complete list of hg19 chromosome names.
- The 9 haplotype chromosomes are as follows:

Name	Accession	UCSC chr Name
HSCHR6_MHC_APD_CTG1	GL000250.1	chr6_apd_hap1
HSCHR6_MHC_COX_CTG1	GL000251.1	chr6_cox_hap2
HSCHR6_MHC_DBB_CTG1	GL000252.1	chr6_dbb_hap3
HSCHR6_MHC_MANN_CTG1	GL000253.1	chr6_mann_hap4
HSCHR6_MHC_MCF_CTG1	GL000254.1	chr6_mcf_hap5
HSCHR6_MHC_QBL_CTG1	GL000255.1	chr6_qbl_hap6
HSCHR6_MHC_SSTO_CTG1	GL000256.1	chr6_ssto_hap7
HSCHR4_1_CTG9	GL000257.1	chr4_ctg9_hap1
HSCHR17_1_CTG5	GL000258.1	chr17_ctg5_hap1

4) Splicing Index (SI) Algorithm

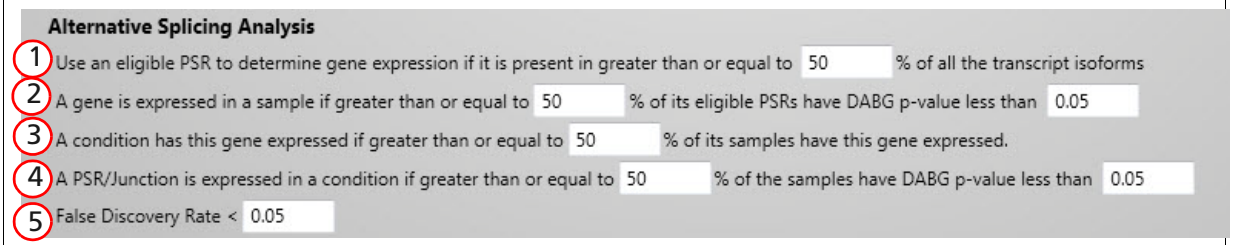
Splicing Index algorithm is a way to measure of how much exon specific expression differs between two conditions after excluding gene level influences. The algorithm first normalizes the exon and junction expression values by the level of gene expression and creates a ratio of normalized signal estimates from one condition relative to another.

Performing an Alternate Splicing Analysis

In the **Preference** window you can customize algorithm parameters by typing values in the text boxes (Figure A.1)

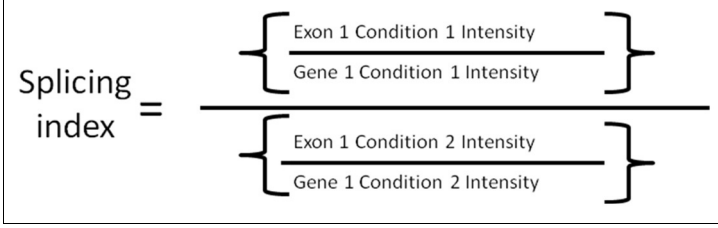
NOTE: Alternate Splicing Analysis is only available for certain arrays. Please contact Affymetrix support regarding which array is supported.

Figure A.1 Configurable Parameters 1-5



The Splicing Index algorithm compares normalized signal estimates from one condition to another. See the equation below: (Figure A.2)

Figure A.2 Splicing Index Algorithm



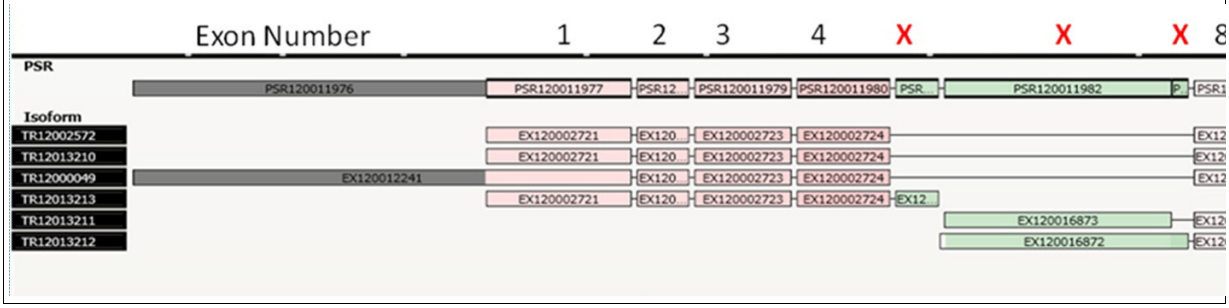
In order for Transcriptome Analysis Console to perform a Splicing Index, two key criteria must be met. They are as follows:

1. **Criteria 1:** A Transcript Cluster gene must be expressed in both conditions. Also, for each condition, you need to determine whether a gene is expressed or not.

- Configurable Parameter **3**: A gene can be considered expressed in a condition (if it meets the criteria specified in parameter 3. As an example, if at least 50% of the samples are expressed, then this gene is expressed in this condition).
- Configurable Parameter **2**: You must determine whether a gene is expressed by looking at the DABG p-values for all the eligible exons (PSRs). As an example, at least 50% of eligible PSRs must be expressed (DABG $p < 0.05$) for the gene to be considered expressed.

- Configurable Parameter **1**: The way to decide whether a PSR is eligible, is to see if it presents at least 50% of all the transcript isoforms for that gene. Only PSRs 1, 2, 3, 4, 8 are considered as eligible PSRs in this gene, as shown in the example below. (Figure A.3)

Figure A.3 Configurable Parameters



2. **Criteria 2:** A PSR or Junction can only be analyzed by Splicing Index if it expresses in at least one condition

- Configurable Parameter **4**: To decide whether a PSR or junction is expressed in a condition, you need (as an example) to check the DABG p-values (from all samples in that condition) to see whether>=50% of samples have DABG p < 0.05.
- After a gene and PSRs/junctions meet the criteria above - normalization and comparison can begin.
- In order to perform normalization, gene intensity needs to be calculated. For a particular gene, the gene intensity for each sample is calculated using the Tukey's Biweight average for all the eligible exons (PSRs) intensities in that gene.
- Next, normalize each PSR or junction intensity using the gene intensity of that sample. (Figure A.2)
- Normalized intensities from Condition1 is compared to normalized intensities from condition 2 using One-way Between-Subject ANOVA for the PSRs and junctions within a gene.
- Configurable Parameter **5**:After running ANOVA, multi-testing correction is performed using Benjamini-Hochberg Step-Up FDR-controlling procedure for all the expressed genes and expressed PSRs/Junctions (expressed in at least one condition.

NOTE: By default, the Alpha level is set as 0.05 in Parameter 5 (False Discovery Rate field).

- For more information, go to:

http://media.affymetrix.com/support/technical/whitepapers/exon_alt_transcript_analysis_whitepaper.pdf

5) Benjamini-Hochberg Step-Up FDR-controlling Procedure

FDR control is a statistical method used in multiple hypothesis testing to correct for multiple comparisons.

- FDR is controlled at certain alpha level (default 0.05 in all result tables). This means that the expected proportion of rejections that are in error is less than alpha. Alpha level can be customized in the result tables by changing the default filtering criteria in the FDR p-value column (0.05) to something else.
- Benjamini, Yoav; Hochberg, Yosef (1995). “Controlling the false discovery rate: a practical and powerful approach to multiple testing”. Journal of the Royal Statistical Society, Series B (Methodological) 57 (1): 289-300. MR 1325392.

6) Fold Change

- Fold change is a number describing how much the signal changes from an initial condition group to a final condition group.
- Fold changes are represented in linear space.

7) Hierarchical Clustering

- Clustering is performed on both probe sets and CHP files.
- Distance metric used between objects is the Euclidean distance.
- Distances between clusters of objects are computed using the complete linkage method (maximum distance between a pair of objects in the two clusters).
- Results are displayed in a heat map and dendrogram.